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Isolation and Molecular Characterization of The Pathogens *Trichoderma harzianum* and Pseudomonas *tolaasii* on the Edible Mushroom *Agaricus bisporus* and Evaluation of Some Desert Plant Extracts for Control Them

ABSTRACT

Seven isolates of Pseudomonas bacteria were isolated from the casing soil and 11 fungal isolates from mushroom farm residues in the mushroom production farm - College of Agriculture , Tikrit University. According to the pathogenicity test, the highest infection rate was recorded by the bacterial isolate 1077, It was diagnosed to the species level as Pseudomonas tolaasii according to the nucleotide sequence of the 16S rRNA gene. It was registered globally in the NCBI website within the accession number MW085029.1, this record is the first for this bacteria in Iraq, while the fungal isolate 1076 showed the highest pathogenicity and this isolate was diagnosed phenotypically to the level of the genus Trichoderma and diagnosed to the level of species T. harzianum according to the nucleotide sequences of the 5.8S rRNA gene and registered globally in the NCBI website within the accession number MW147763.1. In studying the effect of treatments of alcoholic extracts of Atriplex tatarica and Haloxylon salicornicum and their powder in the presence of the pathogenic fungus T. harzianum (Th) and the pathogenic bacteria P. tolaasii (P.t) on the morphological characteristics, infection rate, productivity and quality of mushroom Agaricus bisporus, the results showed that the pathogens had no effect on the most of the morphological characteristics, while the treatment (A. tatarica and H.salicornicum extracts 4 mg/ml + P. tolaasii + T. harzianum) was the best treatment that increased the productivity of the A. bisporus, reduced the severity and rate of infection, and improved of some qualitative characteristics.

INTRODUCTION

The white edible mushroom *A.bisporus* is one of fleshy fungi, which is characterized by its high nutritional value because it contains a high percentage of protein, mineral salts and vitamins that exceeds most types of vegetables, and is characterized by a good smell, taste and flavor distinct from many other food crops (Fekadu,2015), *A.bisporus* belongs to the basidiomycetes that contain a number of an edible mushroom, they are widely spread and known to human since ancient times, in addition to some developed countries such as Japan, European countries and the United States, these countries established industries based on the cultivation of high nutritional mushrooms for the purpose of their consumption as a healthy and medicinal food for humans as well as many medicinal benefits (Kimatu et al., 2017). Ahmad and Hassan, (2015) reported that addition of some organic materials led to an increase in the productivity of mushroom *A.bisporus* and an increase in some qualitative parameters such as antioxidant activity.

The yield of *A.bisporus* is exposed to many diseases that lead to a decrease in its productivity of fruiting bodies and a short shelf life, due to the infection by pathogens, including bacteria that

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cause bacterial spotting disease caused by some species of *Pseudomonas* (Sante, 2011; Nathaniel et al. ,2020), green rot disease, which is caused by the fungus *Trichoderma* spp., this disease appeared in the early nineties of the last century in mushroom farms in North American regions and led to economic losses of more than 30 Million dollars (Hatvani et al., 2007). Research also reported that many global mushroom production farms recorded varying infections of these two diseases, which led to a decrease in its productivity in quantity and quality (Colavolpe et al., 2014: Tanvi et al., 2020). The plants *Atriplex tatarica* and *Haloxylon salicornicum* are among the weeds that are found in most regions of Iraq, especially in the western Anbar desert, which have not been used in an applied way, so the current study aimed to take advantage of these plants in solving one of the most important problems of cultivation of *A. bisporus* which includes control of green rot and brown spot diseases, in addition to evaluating the effectiveness of extracts and powders of *A. tatarica* and *H. salicornicum* in the growth of *A. bisporus* and the production of its fruiting bodies.

MATERIALS AND METHODS

Isolation of bacteria

Samples of the old *A. bisporus* casing soils were collected from the mushroom farm - Tikrit University. They were isolated by a weight of 10 g of casing soil and placed in 90 ml of sterile distilled water to obtain a dilution of 10^{-1}. A series of dilutions were prepared from it up to a dilution of 10^{-6}, 1 ml of the last dilution was taken and placed in a Petri dish, then the nutrient agar medium was poured (sterilized and cooled to 40°C) and mixed well to homogenize the bacteria cells. Then the dishes were incubated at 25°C for 24 hours. The colonies of isolates were purified on the nutrient agar slant according to the same conditions above, then kept in the refrigerator at 4° C until use. The primary distinction was made between the different isolates according to the phenotypic characteristics of their developing colonies in terms of texture, color and size.

Isolation the fungus

Samples of the spent compost used for the cultivation of *A. bisporus* were collected from a previous production cycle of the mushroom farm - Tikrit University. 10 g of the spent compost placed in 90 ml of sterile distilled water to obtain a dilution of 10^{-1}. A series of dilutions were prepared from it, until the dilution 10^{-6}, 1 ml of the last dilution was taken and placed in a Petri dish, then the PDA medium (sterilized and cooled to 40°C) was poured and mixed well to homogenize the reproductive units of the fungi. Then the dishes were incubated at 25°C for 72 hours. The colonies were purified on PDA slants according to the same conditions above, and then kept in the refrigerator at 4°C until use. The primary distinction was made between the different isolates according to the phenotypic characteristics of their developing colonies in terms of texture, color and size, as well as the microscopic characteristics of fungi such as the sepetation of fungal hyphae conidiophore shape and shapes, size, number of cells and colors of conidia(Hassan and Ahmed, 2015).

Estimation of pathogenicity

The pathogenicity of the bacterial and fungal isolates was estimated by methods described by Shafeeq et al. (2021). The most pathogenic bacterium and fungus were molecularly identified.

Molecular Diagnostics

The bacterial isolate and the fungal isolate that gave the highest pathogenicity on the fruiting bodies of *A.bisporus* were diagnosed to the species level according to the molecular method based on the analysis of nucleotide sequences of the 16S rRNA gene for bacteria and the gene 5.8 S rRNA for the fungus.

Genomic DNA Isolation

A swab (100 mg) of a newly grown colony was used for each of the bacteria and fungi isolates separately, and the genomic DNA was extracted using the ready-made ZR Fungal / Bacterial / Yeast DNA Mini Prep TM kit supplied by ZR American company. The genomic DNA was extracted according to the manufacturer's instructions.

Polymerase chain reaction (PCR)

The 16S rRNA gene and the 5.8S rRNA gene were duplicated using PCR technology with the use of the primer pair, the universal primers, whose sequences are shown in Table (1) to determine the selected isolates of the ITS region supplied from (Integrated DNA Technologies company, Canada).

Table (1): the nucleotide sequences of bacterial and fungal primers

Reference	molecular size (base pair)	The nucleotide sequences	Primer	
‹et al 2013) (Miller	1250	5'- AGAGTTTGATCCTGGCTCAG- 3'	Forward	Bacterial primer
		5'- GGTTACCTTGTTACGACTT- 3’	Reverse	
‹et al 1990) (White	650	5′- TCCGTAGGTGAACCTGCGG -3′	Forward	Fungal primer
		5′ TCCTCCGCTTATTGATATGC-3′	Reverse	

Procedure and preparation of the PCR reaction

This technique was used to amplify the ITS region in DNA by the components below and depending on the leaflet attached to the Premix reaction mix (i-Taq DNA Polymerase at 5U/µl, DNTPS at 2.5Mm, Reaction buffer (10X) at 1X, Gel Ioading buffer at 1X. The components of the reaction mixture with their concentrations were final volume of 25 µl as follows: Taq PCR PreMix at a concentration of 5 µl, Forward primer at a concentration of 10 picomol/ml (1 µl), Reverse primer at a concentration of 10 picomol/ml (1 µl), DNA at a concentration of 1.5 µl, Distill water (16.5 µl).

Agarose gel electrophoresis

Applied Biosystem Gene-amp PCR System 9700 Thermal Polymerization Device was used in the process of amplification of the mentioned gene, table (2) showed the standard reaction conditions. The PCR product was separated using electrophoresis on agarose gel (1.5%), then the genomic DNA bands were shown using UV light with a length of wavelength (302nm) after treatment with the dye (Intron Korea red stain).

Table (2): polymerase chain reaction conditions

Phase	Tm(C)	Time	No.of cycle
Initial Denaturation	95 ˚C	5min	1 cycle
Denaturation	95 ˚C	45sec	35 cycle
Annealing	58 ˚C	45sec	
Extension -1	72 ˚C	45sec	
Extension -2	72 ˚C	7min	1 Cycle

Nucleotide sequencing analysis

The nucleotide sequences of the PCR amplified gene were determined after obtaining the 16S rRNA and 5.8S rRNA amplifications directly by sending a volume of 25 µl of PCR product and a volume of 10 µl (10 picomole concentration) of each primer to the Korean company Biotechnology Lab (Applied Biosystem 3730XL, DNA Sequencer device used). The results were compared using a web-based computer program called Basic Local Alignment Search Tool (BLAST) with the database at the National Center for Biotechnology Information (NCBI), which matches the nucleotide sequences of the studied genes with respect to bacterial and fungal isolates placed in the search and knows their species according to the match in the aforementioned database , After completing the identification according to the percentage of similarity with the global strains, the bacterial and fungal isolates were registered globally in the NCBI website

Preparation of plant extracts

The largest quantity of newly-grown plants used in the study was collected, *A. tatarica* was collected from the main site of Anbar University on 30/8/2020 and *H. salicornicum* was collected on 16/9/2020, then dried until its weight stabilized, and after complete drying, it was ground in the electric mill until it became a powder then 20 g of the powder of each plant transferred in a volumetric flask and add to it 100 ml of sterile distilled water (for aqueous extract) and 100 ml of

80% ethanol was added (for alcoholic extract), then both extracts were placed in a shacking incubator at a speed of 120 rpm for 24 hours, then the extracts were filtered using a three-layer gauze cloth, then filtered with Whatman no.1 filter paper. Both extracts were concentrated in a Rotary evaporator at 40°C to get rid of the largest amount of solvent, then the concentrated extract was dried at 40°C (Shafeeq et al. 2021).

Mushroom production experiment

Preparation of pathogens suspensions

For preparation of the pathogenic fungus *T.harzianum* and the pathogenic bacteria *P.tolaasii*, 10 ml of sterile distilled water was added to each fresh pathogens colonies, then the bacterial cells and the fungal colony forming units (CFU) were harvested. then the number was adjusted to 10^8 CFU/ml by cell counting slide.

Spawn Preparation

Spawn of the fungal strain (A15) *A. bisporus* was prepared by transferring a 1 cm^2 piece of fungal colony to glass bottles filled with sterilized wheat grains according to the method (Hassan and Mahmoud, 2003).

Preparation of compost

The compost was prepared and pasteurized according to the standard method mentioned by Hassan et al. (2002).

Incubation and growth

The process of inoculation was carried out for the pasteurized fermented manure after it was packaged in nylon bags measuring 60 cm long, 40 cm wide, and 20 cm high, at a rate of 18 kg per bag. The inoculum was added between layers of manure, sprinkled with 1.5-2%, and mixed homogeneously. The inoculation process included 72 replicates of the medium, incubated at 25 °C, with a relative humidity of 85% (Hassan et al., 2014).

Experiment treatments

Twenty-one combinations of plant powders and extracts of *A.tatarica* and *H. salicornicum* were used to study their effect on the bacterial and fungal pathogens. Table (3) shows the combinations used in the experiment.

Table (3): Treatments Combinations

Treatments combinations
Control
A.tatarica extract (4 mg/ml) with the compost
A.tatarica powder 1% with the compost
A.tatarica powder 2% with the compost
The pathogenic fungus *T. harzianum* (T.h)
A.tatarica extract (4 mg/ml) +*T.h* .
A.tatarica powder 1% +*T.h* .
A.tatarica powder 2% +*T.h* .
H. salicornicum extract (4 mg/ml) with the casing
H. salicornicum powder 1% with the casing
H. salicornicum powder 2% with the casing
Pathogenic bacteria *P. tolassi* (P.t)
H. salicornicum extract (4 mg/ml) + *P.t* .
H. salicornicum powder 1% + *P.t* .
H. salicornicum powder 2% + *P.t* .
A.tatarica + *H. salicornicum* extract 1:1
A.tatarica + *H. salicornicum* powder 1%
A.tatarica + *H. salicornicum* powder 2%
T.h + *P. t*
A.tatarica and *H. salicornicum* extracts (4 mg/ml) + T.h + P. t
A.tatarica and *H. salicornicum* powders (1%) + T.h + P. t
A.tatarica and *H. salicornicum* powders (2%) + T.h + P. t

The pathogenic fungus *T. harzianum* was treated by spraying directly on the compost before placing the casing soil at a rate of 100 ml for each replicate. The pathogenic bacteria were treated by spray after applying the casing soil at a rate of 100 ml for each replicate. Alcoholic extracts were sprayed at a concentration of 4 mg/ ml in the same method, while powders were added at the rate of 1 and 2% in the casing layer, after covering for a period of 7 days, the temperature was reduced to 16°C while the moisture was raised to 85-90% for the purpose of stimulating the fruiting (Hassan et al., 2014).

Measurements

Measurements were made for phenotypic, productivity, and quality traits, and infection indicators, up to only three harvests of mushroom yield.

Phenotypic traits

The dimensions of the fruit bodies, which included the length of the stem and the thickness of the cap, were measured using a ruler and the diameter of the stem and cap using (Ruler verni).

Infection indicators

Estimation of infection rate and severity of green rot disease

The infection rate was estimated based on the number of fruiting bodies that showed symptoms of green rot disease caused by the fungus *T. harzianum*:

Infection rate (%) = number of infected fruit bodies / total number * 100

In order to estimate the severity of the infection, the following pathological index was adopted, which was developed in this study and which is based on the pathological symptoms (a healthy fruit body (white color) = 0, partially colored fruit body (green/brown) = 1, fully colored fruit body = 2, fully colored fruit body + some deformation = 3, colored and deformed fruiting body with complete rot = 4). The the severity of infection was estimated for all treatments according to (Mckinney, 1923) equation:

Infection severity =

$$\frac{Sum(The\ number\ of\ infected\ fruits\ at\ degree\ 0*0 + ...+\ the\ number\ of\ infected\ fruits\ at\ degree\ 6 * 6)}{Total\ number\ of\ fruits * highest\ score} 100*$$

Estimation of infection rate and severity of brown spotting disease

The infection rate was estimated based on the number of fruiting bodies that showed symptoms of brown spots caused by *P.tolaasii*. The infection rate was calculated as in the case of fungal rot disease, while the following symptoms were adopted to calculate the severity of infection: (A healthy fruit body (white color = 0, body Partially colored (pale brown) fruiting = 1, completely pale brown colored fruiting body = 2, dark brown colored fruiting body without bacterial oozes = 3, fully colored fruiting body with bacterial oozes= 4). Then the severity of infestation was estimated for all treatments according to the equation (Mckinney, 1923) mentioned above.

Productive traits

The productivity was estimated for each replicate for three flashes. The productivity included number of fruit bodies and weight of the mushroom yield for each harvest. Then the biological efficiency was estimated, which expresses a relationship with the dry compost weight:

% Biological efficiency (B.E = fresh weight of the fruit bodies (kg) / dry weight of the medium (kg) x 100 (1985, Royse). Finally, the yield was calculated using the production formula per unit area (kg fresh mushrooms / m^2).

Estimation of some qualitative characteristics

Determination of ash, protein, moisture and dry weight of fruiting bodies according to the methods mentioned in AOAC (2002).

Statistical analysis

The study experiments were implemented using the Completely Randomized Design (CRD) and analysis of variance was conducted using the SPSS program, the comparison of means was conducted according to the Least Significant Deference (LSD) test at the probability level of 0.05 (Al-Rawi and Khalaf Allah, 1980).

RESULTS AND DISCUSSION

The molecular diagnosis of isolate 1077 was based on the level of *P. tolaasii* by nucleotide sequencing of 16S rRNA gene. Figure (1-A) shows the bands resulting from the electrophoresis of genomic DNA, (1-B) shows the electrophoresis of the PCR product using the universal primer for 16S rRNA gene amplification. The electrophoresis results show the appearance of bands of the size of 1250 base pairs, which is evidence of the accuracy of the PCR test.

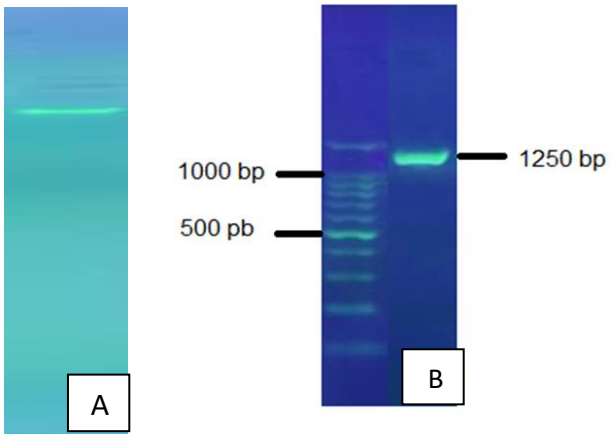

Figure (1) Electrophoresis of the genomic DNA of *Pseudomonas* isolate 1077 (A) Electrophoresis of the product of the polymerase chain reaction using a specialized primer to amplify the 16S rRNA gene for Pseudomonas isolate 1077 (B)

Table (4) shows the percentages of similarity of bacterial isolate 1077 and their conformity with the highest percentage of match with the bacterial strain *P. tolaasii* registered globally and its global numbers in the genetic bank database, as the similarity percentage reached 99.49%. The bacteria *P. tolaasii* (isolate 1077) was recorded at the NCBI site under the accession number MW085029.1 and this record is the first for this bacteria in Iraq.

Table (4): Molecular diagnosis of *P. tolaasii* isolate (1077) according to the highest percentage of matching 16S rRNA gene sequences with *P. tolaasii* strain in the World Genetic Bank

Accession number for bacterial isolates identified in this study	Similarity %	Country	Accession number	The most compatible species of bacteria	Isolation of bacteria code
MW085029.1	99.49	Indian	JF800925.1	*Pseudomonas tolaasii*	1077

In figure (2-A) showed the bands resulting from the electrophoresis of genomic DNA, while (2-B) showed the electrophoresis of the product of the polymerase chain reaction using the universal primer for gene amplification 5.8S rRNA. The results show the presence a band with a size of 650 base pairs, which indicates the accuracy of the procedure this test. Table (5) shows the molecular diagnosis of the fungus *T. harzianum* (1076) and its conformity with the highest percentage of similarity with the strain of the fungus *T. harzianum* registered globally and its global numbers in the genetic bank database, where the similarity rate reached 99.21%, the fungus isolate recorded *T. harzianum* (1076) on the NCBI website under the accession number MW147763.1.

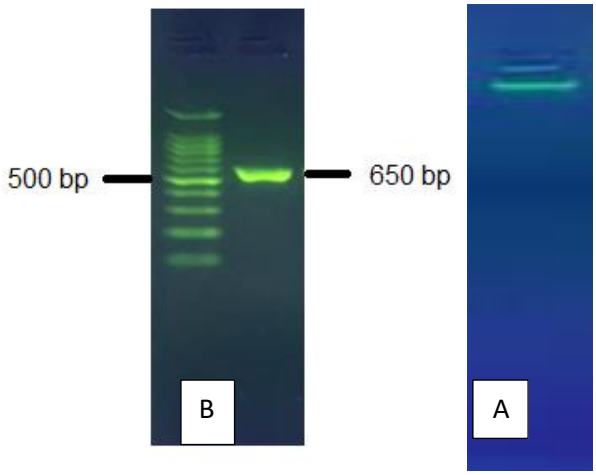

Figure 2. Electrophoresis of the genomic DNA of the *T. harzianum* isolate 1076 (A). Electrophoresis of the polymerase chain reaction using a universal primer to amplify the 5.8S rRNA gene (B)

Table (5): Molecular diagnosis of *T. harzianum* isolate (1076) according to the percentage of identical 5.8S rRNA gene sequences with the strain of *T. harzianum* in the World Genetic Bank

Accession number for fungal isolates identified in this study	Similarity %	Country	Accession number	The most compatible species of fungi	Isolation of fungal code	No.
MW147763.1	99.21	Pakistan	MH128129.1	*Trichoderma harzianum*	1076	1

Although the isolates of bacteria and fungi isolated in this study are identical within the studied nucleotide sequences by 99.21- 99.49% with the internationally recorded bacteria and fungi strains, they can be considered genetically different isolates because there is no match (100%), and this can be attributed to the occurrence of many Genetic variations due to environmental factors and continuous spraying of chemical pesticides or environmental pollution factors that spread whether at the level of soil or air pollution, which leads to the occurrence of genetic mutations and thus genetic variation occurs that is reflected in the physiological factors of these species (Martins et al., 2013; Cadet and Wagner, 2014). Alkurtany et al. (2018) reported that molecular identification by using the primers 16SPSEfluF and 16SPSEfluR for the amplification of the gene 16S rRNA using PCR technique, gave accurate results in diagnosis of *P. fluorescens*.

Effect of extracts and powders of *A. tatarica* and *H.salicornicum* on the phenotypic characteristics of *A. bisporus* under conditions of infection with pathogens *T. harzianum* and *P. tolassi*.

The results listed in Table (6) show the effect of extracts and powders of *A. tatarica* and *H.salicornicum* on the phenotypic characteristics of *A. bisporus* under the conditions of infection with the pathogenic fungus *T. harzianum* and the pathogenic bacteria *P. tolassi*. The results showed the stem length in all treatments were within the standard specifications, the highest stem length in the treatment (*A. tatarica* and *H.salicornicum* extract 4 mg/ml + P.t + T.h), which reached 7.00 cm, followed by (*A. tatarica* and *H.salicornicum* powder 2% + P.t + T.h) and (*A. tatarica* and *H.salicornicum* powder 1% + P.t + Th) which reached 6.97 and 6.96 cm, respectively, while the lowest stem length was in the treatment (*H.salicornicum* powder 2% + P.t) which reached 5.63 cm. For the highest stem diameter, the treatment (*A. tatarica* extract + *H.salicornicum* extract 1:1) was significantly superior on other treatments, which was 4.45 cm, followed by (*A. tatarica* powder 2% + *H.salicornicum* powder 2%), which reached 4.38 cm, while the lowest stem diameter was recorded in the treatment (P.t + T.h) which was 3.07 cm, followed by (pathogenic fungus T.h)

which was 3.16 cm. The results showed the highest cap diameter was in (***A.*** *tatarica* extract + *H.salicornicum* extract 1:1) it reached 11.54 cm, followed by (***A.*** *tatarica* powder 2% + *H.salicornicum* powder 2%) which reached 11.47 cm, while the lowest cap diameter was 9.55 cm in the treatment (P.t + T.h), followed by (pathogenic fungus T.h) which reached 9.69 cm. For the cap thickness, the highest cap thickness was in (***A.*** *tatarica* extract + *H.salicornicum* extract 1:1), which amounted to 6.41 cm, followed by (***A.*** *tatarica* powder 2% + *H.salicornicum* powder 2%), which reached 6.36 cm, while the lowest cap thickness was in the treatment (Th + Pt), which was t 4.51 cm.

Table (6): Effect of extracts and powders of *A.tatarica* and *H. salicornicum* on the phenotypic characteristics of *A. bisporus* under conditions of the pathogens *T. harzianum* and *P. tolassi*

Cap thickness cm	Cap diameter cm	Stem diameter cm	Stem length cm	Treatments codes
6.06	11.21	3.87	6.19	Control
6.26	11.32	4.23	6.25	*A.tatarica* extract (4 mg/ml)
6.07	11.11	3.99	6.07	*A.tatarica* powder 1%
6.16	11.20	4.14	6.19	*A.tatarica* powder 2%
5.01	9.69	3.16	6.85	*T. harzianum* (T.h)
5.42	10.47	3.54	6.87	*A.tatarica* extract (4 mg/ml) +*T.h* .
5.22	10.30	3.36	6.81	*A.tatarica* powder 1% +*T.h* .
5.31	10.43	3.45	6.81	*A.tatarica* powder 2% +*T.h* .
6.20	11.28	4.17	6.24	*H. salicornicum* extract (4 mg/ml)
5.99	11.01	3.93	6.07	*H. salicornicum* powder 1%
5.92	10.97	3.98	5.67	*H. salicornicum* powder 2%
5.13	9.88	3.28	6.87	*P. tolassi* (P.t)
5.91	10.93	3.84	6.03	*H. salicornicum* extract (4 mg/ml) + *P.t* .
5.80	10.83	3.78	5.98	*H. salicornicum* powder 1% + *P.t* .
5.88	10.91	3.79	5.63	*H. salicornicum* powder 2% + *P.t* .
6.41	11.54	4.45	6.26	*A.tatarica* + *H. salicornicum* extract 1:1
6.28	11.38	4.30	6.19	*A.tatarica* + *H. salicornicum* powder 1%
6.36	11.47	4.38	6.24	*A.tatarica* + *H. salicornicum* powder 2%
4.51	9.55	3.07	6.92	*T.h* + *P. t*
5.72	10.77	3.76	7.00	*A.tatarica* + *H. salicornicum* extracts (4 mg/ml) + T.h + P. t
5.55	10.57	3.60	6.96	*A.tatarica* + *H. salicornicum* powders (1%) + T.h + P. t
5.63	10.65	3.67	6.97	*A.tatarica* +*H. salicornicum* powders (2%) + T.h + P. t
0.25	0.40	0.28	0.41	$LSD_{0.05}$

Although the phenotypic data recorded during this study were within the standard specifications, there are some differences in stem length, diameter, cap thickness and diameter, and this may be due to the presence of active substances in plant extracts and powders represented by total alkaloids, tannins, saponins, glycosides, phenols and flavonoids, which had inhibitory role of diseases and this variation in phenotypic characteristics was evident in the listed data in the table (6). This difference may be due to the content and concentrations of active substances in alcoholic extracts were higher proportions than in water, as well as the possibility that the raw powder contains other compounds such as proteins, carbohydrates, vitamins and mineral salts that helped provide nutrients within the culture medium and increase its effectiveness towards providing the

basic materials for the edible mushroom *A.bisporus* in addition its inhibitory effect on the pathogens that compete with the edible mushroom (Shafeeq et al. 2021) .

Effect of extracts and powders of *A. tatarica* and *H.salicornicum* on the infection of *A. bisporus* with pathogens *T. harzianum* and *P. tolassi*.

Table (7) showed the effect of *A. tatarica* and *H.salicornicum* extracts and powders on the infection of the *A. bisporus* with the pathogens *T. harzianum* and *P. tolassi*, as the highest infection rate reached in the treatment (T.h + P.t) reached 70.06%, followed by the (pathogenic fungus T.h) it reached 55.35%, while the lowest rates of infection was in (*A. tatarica* + *H.salicornicum* powder 2%) amounted to 0.27%, followed by treatment (*A. tatarica* powder 2%), which was 0.36%. For the level of infection severity, the highest severity of infection was recorded in the treatment (T.h+P.t) which was 72.76%, followed by the treatment of (pathogenic fungus T.h), which was 60.36%, and the lowest infection severity was in (*A. tatarica* + Haloxylon powder 2%) which was 0.34%, followed by both treatments (*A. tatarica* + *H.salicornicum* powder 1%) and (*A. tatarica* powder 2%) which was 0.37%. Our results are consistent with studies in this field, as they are in agreement with the study of Hassan and Ahmed,(2015) who stated that the manufacture of compost from different plants led to the inhibition of the pathogenic fungus *Macrophomina phaseolina* on corn.

The study showed a difference in the level of severity and incidence of infection as the alcoholic extracts had a high inhibition of the pathogens used in the study, the inhibitory effect was clear on the treatment level and no symptoms of infection appeared. This may be due to the effectiveness of the solubility of the active substances found in alcoholic extract, represented by total alkaloids, tannins, saponins, flaglycosides, phenols and flavonoids, as their inhibitory effect (Hassan and Ahmed, 2015). For the severe infection at the level of the combined treatment of both pathogens, an infection was recorded in the combined treatment of both pathogens at higher rates than in the treatment containing one pathogen, may be due to the chance of competition against the *A.bisporus*, which leads to the infection and rotting of the fruit bodies of the *A.bisporus* faster than the rest of the treatments that contain each pathogen separately.

Table (7): Effect of *A. tatarica* and *H.salicornicum* extracts and powders on infection of *A. bisporus* with pathogens *T. harzianum* and bacteria *P. tolassi*

Infection severity (%)	Infection rate (%)	Treatments
0.34	0.54	Control
0.00	0.00	*A.tatarica* extract (4 mg/ml)
0.51	0.44	*A.tatarica* powder 1%
0.37	0.36	*A.tatarica* powder 2%
60.36	55.35	*T. harzianum* (T.h)
20.05	18.76	*A.tatarica* extract (4 mg/ml) +*T.h* .
30.77	30.15	*A.tatarica* powder 1% +*T.h* .
27.63	27.09	*A.tatarica* powder 2% +*T.h* .
0.00	0.00	*H. salicornicum* extract (4 mg/ml)
0.43	0.42	*H. salicornicum* powder 1%
0.39	0.38	*H. salicornicum* powder 2%
56.56	52.63	*P. tolassi* (P.t)
16.51	16.78	*H. salicornicum* extract (4 mg/ml) + *P.t* .
25.69	21.41	*H. salicornicum* powder 1% + *P.t* .
21.97	22.21	*H. salicornicum* powder 2% + *P.t* .
0.00	0.00	*A.tatarica* + *H. salicornicum* extract 1:1
0.37	0.37	*A.tatarica* + *H. salicornicum* powder 1%
0.34	0.27	*A.tatarica* + *H. salicornicum* powder 2%
72.76	70.06	*T.h* + *P. t*
21.00	20.35	*A.tatarica* + *H. salicornicum* extracts (4 mg/ml) + T.h + P. t
32.96	32.74	*A.tatarica* + *H. salicornicum* powders (1%) + T.h + P. t
27.95	26.93	*A.tatarica* + *H. salicornicum* powders (2%) + T.h + P. t
2.50	2.23	$LSD_{0.05}$

Effect of extracts and powders of *A. tatarica* and *H.salicornicum* on the productive traits of *A. bisporus* under conditions of infection with pathogens *T. harzianum* and *P. tolassi*.

The results in table (8) showed the effect of *A. tatarica* and *H.salicornicum* extracts and powders on the productive traits of *A. bisporus* under the conditions of infection with *T. harzianum* and *P. tolassi*, as the highest number of fruiting bodies was recorded in (*A. tatarica* powder 2% + *H.salicornicum* powder 2%) it reached 153.64, followed by treatment (*A. tatarica* + *H.salicornicum* extract 1:1), which reached to 147.85, while the lowest number of fruiting bodies was in the treatment (T.h + P.t) resulting in 74.15, followed by the treatment of (pathogenic fungus T.h), which reached to 85.10. At the level of the weight of the fruit bodies, the highest weight was recorded in the treatment (*A. tatarica* + *H.salicornicum* powder *2*%) amounted to 4667.63 g, followed by a treatment (*A. tatarica* extract + *H.salicornicum* extract 1:1), which was 4609.30 g, while the lowest weight was in the treatment (T.h + P.t), which was 1706.61 g. In biological efficiency, the highest value was in the treatment (*A. tatarica* + *H.salicornicum* powder *2*%) amounted to 77.78%, followed by the treatment (*Atriplex* extract + *H.salicornicum* extract 1:1), which was 76.81%, and the lowest biological efficiency was recorded in the treatment (T.h + P.t), which was 28.43%. The highest production (in term Kg fruit bodies/m^2) was in (*A. tatarica* powder 2% + *H.salicornicum* powder 2%), reached to 19.59 kg / m^2, followed by (*A. tatarica* extract + *H.salicornicum* extract 1:1), which was 19.35 kg / m^2, while the lowest production was recorded in the treatment (T.h + Pt), which amounted to 7.14 kg / m^2.

Table (8): Effect of *A. tatarica and H.salicornicum* plant extracts and powders on the productive characteristics of *A. bisporus* under conditions of infection with pathogenic fungus *T. harzianum* and pathogenic bacteria *P. tolassi*

Production (kg / m^2)	Bio efficiency(%)	Fruiting body weight (gm)	The number of fruiting bodies	Treatments
16.03	63.65	3820.24	135.17	Control
17.96	71.29	4278.38	144.75	*A.tatarica* extract (4 mg/ml)
16.15	64.13	3848.76	138.06	*A.tatarica* powder 1%
17.49	69.44	4167.80	147.01	*A.tatarica* powder 2%
7.51	29.88	1793.51	85.10	*T. harzianum* (T.h)
14.22	56.45	3387.93	130.32	*A.tatarica* extract (4 mg/ml) +*T.h* .
13.69	54.39	3263.76	122.56	*A.tatarica* powder 1% +*T.h* .
14.21	56.41	3385.70	133.64	*A.tatarica* powder 2% +*T.h* .
17.03	67.62	4058.09	140.95	*H. salicornicum* extract (4 mg/ml)
16.45	65.31	3919.29	136.33	*H. salicornicum* powder 1%
17.37	68.95	4138.13	143.94	*H. salicornicum* powder 2%
13.80	54.80	3288.45	130.78	*P. tolassi* (P.t)
14.64	58.16	3490.28	136.51	*H. salicornicum* extract (4 mg/ml) + *P.t* .
13.97	55.50	3329.84	131.08	*H. salicornicum* powder 1% + *P.t* .
14.99	59.54	3572.73	139.66	*H. salicornicum* powder 2% + *P.t* .
19.35	76.81	4609.30	147.85	*A.tatarica* + *H. salicornicum* extract 1:1
18.46	73.33	4400.91	145.95	*A.tatarica* + *H. salicornicum* powder 1%
19.59	77.78	4667.63	153.64	*A.tatarica* + *H. salicornicum* powder 2%
7.14	28.43	1706.61	74.15	*T.h* + *P. t*
13.79	54.82	3290.04	123.83	*A.tatarica* + *H. salicornicum* extracts (4 mg/ml) + T.h + P. t
13.27	52.71	3162.98	115.44	*A.tatarica* + *H. salicornicum* powders (1%) + T.h + P. t
14.04	55.72	3344.29	125.44	*A.tatarica* + *H. salicornicum* powders (2%) + T.h + P. t
1.01	3.25	61.83	3.81	$LSD_{0.05}$

The exploitation of the nutritional components present in the mushroom compost by pathogens may be a cause for the emergence of pathological symptoms on the fruiting bodies of *A.bisporus* and as a result of the secretion of several hydrolytic enzymes caused to the decomposition of the fruiting bodies, this leads to a decrease in their number, weight and biological efficiency, the pathogens also killed the fungal hyphae of *A.bisporus* in the compost and the exploitation of the compost surface area, this results agree with some aspects of the study of Choi et al.,(2012).

Fig. 3 showed the low productivity of *A. bisporus* and rotting symptoms of the green rot disease caused by *T. harzianum*, while fig. 4 showed brown spot symptoms of the pathogen *P.tolaasii* , compared with good productivity and healthy fruit bodies in the best treatment (*A.tatarica* + *H. salicornicum* powder 2%) in fig.5 and control (fig.6).

Figure (3) Productivity of the edible mushroom *A.bisporus* with the presence of rotting symptoms of the green rot disease caused by *T. harzianm*

Figure (4) Productivity of the *A.bisporus* with the presence of brown spot symptoms caused by *P.tolaasii*

Figure (5) Productivity of the *A.bisporus* with the presence of *A. tatarica* and *H.salicornicum* powders (2%) without pathogens

Figure (6) Productivity of control treatments for the *A.bisporus*

Table (9): Effect of extracts and powders of *A. tatarica* and *H.salicornicum* on some qualitative characteristics of *A. bisporus* under conditions of infection with pathogens *T. harzianum* and *P. tolaasii*

Moisture(%)	Protein (%)	Ash (%)	Dry Weight (%)	Treatments
90.6	3.28	8.88	9.40	Control
90.56	3.49	9.03	9.44	*A.tatarica* extract (4 mg/ml)
90.58	3.41	8.85	9.42	*A.tatarica* powder 1%
90.55	3.71	8.96	9.45	*A.tatarica* powder 2%
91.97	2.60	7.05	8.03	*T. harzianum* (T.h)
91.27	3.29	7.81	8.73	*A.tatarica* extract (4 mg/ml) +*T.h* .
91.34	3.39	7.68	8.66	*A.tatarica* powder 1% +*T.h* .
91.3	3.50	7.72	8.70	*A.tatarica* powder 2% +*T.h* .
90.55	3.47	9.0	9.45	*H. salicornicum* extract (4 mg/ml)
90.57	3.34	8.80	9.43	*H. salicornicum* powder 1%
90.54	3.49	8.91	9.46	*H. salicornicum* powder 2%
91.25	2.72	7.69	8.75	*P. tolassi* (P.t)
90.69	3.32	8.63	9.31	*H. salicornicum* extract (4 mg/ml) + *P.t* .
91.02	3.35	8.56	8.98	*H. salicornicum* powder 1% + *P.t* .
90.84	3.39	8.61	9.16	*H. salicornicum* powder 2% + *P.t* .
89.74	3.92	9.11	10.26	*A.tatarica* + *H. salicornicum* extract 1:1
90.48	3.75	9.07	9.52	*A.tatarica* + *H. salicornicum* powder 1%
89.59	4.07	9.32	10.41	*A.tatarica* + *H. salicornicum* powder 2%
92.67	1.73	6.88	7.33	*T.h* + *P. t*
91.09	3.37	8.33	8.91	*A.tatarica* + *H. salicornicum* extracts (4 mg/ml) + T.h + P. t
91.21	3.74	7.88	8.79	*A.tatarica* + *H. salicornicum* powders (1%) + T.h + P. t
91.14	3.83	8.11	8.86	*A.tatarica* + *H. salicornicum* powders (2%) + T.h + P. t
1.03	0.16	0.41	0.38	LSD $_{0.05}$

Effect of extracts and powders of *A. tatarica* and *H.salicornicum* on some qualitative characteristics of *A. bisporus* under conditions of infection with pathogens *T. harzianum* and *P. tolassi*.

The results of table (9) showed the highest dry weight was in (*A. tatarica* powder 2% with *H.salicornicum* powder 2%) it reached 10.41 %, followed by (*A. tatarica* extract with *H.salicornicum* extract 1:1), which was 10.26 %, while lowest dry weight recorded in the treatment

(T.h + Pt), reached to 7.33%, followed by (the pathogenic fungus *T. h*) which was 8.03%. The highest percentage of ash was recorded in treatment (*A. tatarica* powder 2% with *H.salicornicum* powder 2%), which amounted to 9.32%, followed by (*A. tatarica* extract with *H.salicornicum* extract 1:1), which was 9.11%, while the lowest ash percentage was recorded in the treatment (T.h + Pt), resulting in 6.88%. The results also showed the highest protein content was recorded in treatment (*A. tatarica* + *H.salicornicum* powder *2*%) amounted to 4.07 %, followed by (*A. tatarica* with *H.salicornicum* extract 1:1), which was 3.92%, while the lowest protein recorded in the treatment (T.h + Pt), it reached 1.73%, followed by (pathogenic fungus *T.*) which was 2.60%. The moisture content in fruit bodies was higher in the treatment (T.h + Pt), reached 92.67%, followed by (*T. h* treatment), which reached 91.97%, while the lowest moisture was 89.59% in the treatment (*A. tatarica* + *H.salicornicum* powder 2%).

The study showed the extracts and powders of *A. tatarica* and *H.salicornicum* have an important role in the improvement of *A. bisporus* qualitative characteristics under conditions of infection with pathogens *T. harzianum* and *P. tolaasii* , this due to the active substances in the extracts and powders of both plants inhibited the pathogens growth in addition helped in strengthening the compost medium for the *A.bisporus* and perhaps the extracts and powders contain nutrients that may have encouraged the fungus growth, which led to an increase protein and dry matter then increase in mushroom yield, these results were agree with other related studies (Hassan et al., 2002; Hassan and Ahmed, 2015).

CONCLUSION

As a conclusion, desert plants *A. tatarica* and *H. salicornicum* and their alcoholic extracts have proven an important role in inhibiting both pathogens *T. harzianum* and *P. tolaasii*, that infected the edible mushroom *A. bisporus* in addition to promoting the growth of this mushroom, increasing their productivity and improving their quality characteristics.

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العزل والتشخيص الجزيئي للفطر *Trichoderma harzianum* والبكتريا *Pseudomonas tolaasii* وتقييم إمراضيتهما على الفطر الغذائي *Agaricus bisporus* واختبار كفاءة نباتي *Atriplex tatarica* و *Haloxylon salicornicum* في مقاومتها

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الكلمات المفتاحية:
Atriplex tatarica ، Haloxylon salicornicum ، Alcholic extracts.، مرض التبقع البني، مرض التعفن الاخضر، الفطر الغذائي Agaricus bisporus

الخلاصة

تم عزل 7 عزلات من بكتريا *Pseudomonas* من تربة التغطية و11 عزلة فطرية مخلفات مزرعة الفطر في مزرعة انتاج الفطر - كلية الزراعة – جامعة تكريت. وحسب اختـ الإمراضية سجلت اعلى نسبة اصابة من قبل العزلة البكتيرية 1077 وشخصت جزيئيا مستوى النوع *Pseudomonas tolaasii* حسب إختبار التتابع النيوكليوتيدي للجين S rRNA وسجلت عالميا في الموقع NCBI ضمن الرقم العالمي MW085029.1 و التسجيل يعد الأول لهذه البكتريا في العراق ، اما العزلة الفطرية 1076 ابدت اعلى إمراض وشخصت هذه العزلة مظهريا الى مستوى الجنس *Trichoderma* وشخصت الى مستو النوع *T. harzianum* حسب التشخيص الجزيئي المعتمد على التتابعات النيوكليوتيدية لج 5.8S rRNA وسجلت ضمن الرقم العالمي MW147763.1 .درس تأثير المستخلـ الكحولي لنباتي الرغل *Atriplex tatarica* والرمث *Haloxylon salicornicum* ومسحوقهما بوجود الفطر الممرض *T. harzianum* والبكتريا الممرضة *P. tolaasii* الصفات المظهرية والاصابة والانتاجية والنوعية للفطر الغذائي *Agaricus bisporus* ، اظهرت النتائج عدم تأثير الممرضات بمعظم الصفات المظهرية بينما كانت معاملة (مستخلـ الرغل والرمث 4 ملغم / مل + *P. tolaasii+ T. harzianum*) افضل معاملة ادت زيادة انتاجية الفطر وخفضت من شدة ونسبة الاصابة وحسنت من بعض الصفات النوعية .