Evaluation of The Efficiency of Some Desert Plant Extracts in The Growth of Agaricus Bisporus and The Inhibition of Some of Its Pathogens

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Abstract

Seven isolates of Pseudomonas spp. were isolated from the casing soil and 11 fungal isolates were isolated from the spent compost in the Mushroom Farm - College of Agriculture - Tikrit University. According to the pathogenicity test against Agaricus bisporus fruit bodies, the results showed that the highest infection rate was recorded in the bacterial isolate 1077 by 77.45%, while the fungal isolate 1076 showed the highest pathogenicity against Agaricus bisporus fruit bodies by recording the highest infection rate reached to 81.23%. The pathogenic bacteria and fungi isolates were phenotypically identified to the species level as Pseudomonas tolaasii and Trichoderma harzianum, respectively. The results showed the alcoholic extracts of Atriplex tatarica and Haloxylon salicornii were superior in the active substances compared to other extracts, the highest percentage of total alkaloids, tannins, saponins, phenols and flavonoids was 35.14, 44.79, 43.47, 32.05 and 31.66 mg/g in the alcoholic extract of A. tatarica, respectively, while the alcoholic extract of H. salicornium had the highest percentage of glycosides, which amounted to 41.89 mg/g of the extract. The highest efficiency of A. tatarica alcoholic extract in inhibiting the pathogenic fungus T. harzianum, was recorded, as the colony diameter was 3.1 cm in concentration of 4 mg/mL compared to 8.9 cm in the control treatment, while the H. salicornium alcoholic extract was superior in inhibiting the pathogenic bacteria at the same concentration, in which the inhibition zone was 15.67 mm compared to no inhibition in the control treatment. While there was little effect of alcoholic extracts of both plants on the growth of A. bisporus. The results of the dry powders effect for both plants showed that the highest inhibition against the pathogenic fungus T. harzianum was recorded by the A. tatarica powder at 2% concentration, reaching 5.01 cm, while the H. salicornium powder showed the highest inhibition against the bacteria P. tolaasii at the same concentration in which the number of cells reached to 3.23 x 10⁷ cells/mL compared to 6.6 x 10⁷ cells/mL in the control treatment. The results also showed the highest growth of A. bisporus that reached 9 cm for all powdered concentrations of both plants.

Keywords: Alcoholic extracts, Desert plants, Agaricus bisporus, P. tolaasii, T. harzianum.

1. Introduction

Recently, fleshy-edible mushrooms have been known as a special type of food, that pose high level of protein source, their protein content is higher than all vegetables, as the protein percentage ranges from 20-40% on a dry weight basis. This protein contains all essential and non-essential amino acids. Fresh mushrooms contain 3-21% carbohydrates, 3-35% fibers, 2-8% fats and also contain many vitamins and mineral salts [1], in addition to the fact that fleshy mushrooms are of great importance in the food and pharmaceutical industries because they contain antitumors, immunostimulating, cholesterol-lowering, and atherosclerosis agents [2].

Green mold disease caused by the fungus Trichoderma spp. and brown spot disease caused by the bacteria Pseudomonas tolaasii are among the diseases prevalent in A. bisporus production farms, as statistics indicate that green rot disease appeared in the early nineties in mushroom farms in North American regions (Alberta, Ontario, British Columbia and Pennsylvania), and it led to economic losses of more than 30 million dollars. The fungus Trichoderma spp. was identified as the causal agent of this disease [3]. A number of fungal pathogens that infect the yield of edible mushroom A. bisporus were recorded in Iraq by [4], in this study, four fungal pathogens were recorded, where three of them including wet bubble caused by Mycogone perniciosa, Verticillium spot caused by Verticillium fungicola and cobweb mold caused by Dactylilum dendroides infect the mushroom fruit bodies and caused direct losses in its yield, the fourth pathogen related to green moulds Trichoderma viride and T. harzianum, which infect the mushroom mycelium and caused indirect losses in its yield. There are several species of
bacteria related to the genus *Pseudomonas* caused spotting and brown blotching on the fruiting bodies, and the most important conditions for encouraging this disease are humid atmospheres and moderate environmental conditions in temperatures, in addition to contamination of the materials used in mushroom cultivation or the movement of workers in the cultivation rooms without applying general hygiene procedures [5]. Infections of the fruiting bodies with these bacteria lead to a reduction in the quality of the mushroom yield and lose its marketing value. In the case of advanced infections and the availability of appropriate conditions for the causative bacteria, it leads to the linking of the spots with each other and permeating the entire fruit body and losing its qualitative and economic value. Also the risk of infection with this bacterium increases because it is one of the endemic bacteria in the casing soils, and if the appropriate conditions are available, pathogenicity occurs in the fruiting bodies [6].

Research is still focused on the use of multiple methods to increase the production of the *A. bisporus* yield as well as to control its various diseases. One of these methods is to supplement the media with the nutrients of plant extracts and powders that are effective in improving the properties of the medium, so that the nutrients are in the best form preferred by the mycelium and resistance to diseases that affect the fungus instead of chemical pesticides that have negative effects on humans and their ecosystem [7]. The plants *Atriplex* spp. and *Haloxylon* spp. are from the weeds that are found in most areas of Iraq, especially in the Western Anbar Desert, which have not been used in an applied way and for the purpose of benefiting from these plants in solving one of the most important problems of cultivation of the fungus *A. bisporus*, which is the control of the green rot and brown spot diseases. This study aimed to Isolation and identification of green rot disease caused by *Trichoderma* sp and brown spot disease caused by *Pseudomonas* sp bacteria spread in most mushroom production farms in addition to evaluation of the efficiency of extracts and powders of *Atriplex* spp. and *Haloxylon* spp. in inhibiting these pathogens.

2. Materials and Methods

2.1 Isolation of bacterial and fungal pathogens

The bacteria *Pseudomonas* sp was isolated from the fruit bodies of *A. bisporus* (obtained from local markets) which showed symptoms of bacterial spot, while the fungus *Trichoderma* sp. was isolated from the compost of the Mushroom Farm – Tikrit University. The isolation of bacteria was carried out on a King and nutrient agar media, while the isolation of *Trichoderma* sp. was carried out on a medium of potato dextrose agar (PDA) according to the method mentioned by [8].

2.2 Determination of pathogenic bacterial and fungal isolates

2.2.1 Preparation of the suspensions

The bacterial suspensions were prepared after updating their cultivation using the method of diffusion on a nutrient medium to obtain the active colonies at 24 hours old, 10 mL of sterile distilled water was placed for each petri-dish, then the bacteria cells were harvested using a sterile fine brush and collected in a sterile test tube. The number of bacterial cells was adjusted to $1 \times 10^8$ cells/mL using the method of culturing decimal dilutions of this suspension in a nutrient agar medium. The developing colonies were counted after 24 hours. The number of bacteria is calculated by multiplying the number of colonies by the reciprocal of the dilution. Fungal suspension was prepared after updating their culture on PDA medium to obtain active colonies 72 hours old, 10 mL of sterile distilled water was placed for each petridish, then harvested the spores of the fungi using a sterile fine brush and collected the suspension in a sterile test tube, adjusting the number of spores to $10^9$ CFU/ml using the method of culturing decimal dilutions of this suspension in the medium of PDA, the developing colonies were counted after 72 hours. The number of CFU of the fungi is calculated by multiplying the number of colonies by the reciprocal of the dilution [9].

2.2.2 Identification of pathogens

The pathogenic isolates of bacteria were identified to the level of *P. tolaasii* and the fungus to the level of *T. harzianum* according to the Morphological and molecular methods mentioned by [10].

2.2.3 Pathogenicity test

The pathogenicity test of the bacterial isolates (*Pseudomonas* sp.) was performed by spraying the bacterial suspension ($1 \times 10^9$ cells/mL) on the fruits of the fungus *A. bisporus* (which was obtained from the local markets and did not show any symptoms of infection), 50 fruit bodies (at an average weight of 20 g) were sprayed with 50 mL of the bacterial suspension,
other 50 fruit bodies were sprayed with 50 mL of the non-inoculated nutrient broth was sprayed as control. The fruit bodies were incubated at 25 °C for 3 days, then the infection rate was calculated as follows:

\[
\text{Infection rate} = \frac{\text{number of infected fruit bodies}}{\text{total number of fruit bodies}} \times 100
\]

The fruit bodies are returned infected in the event of the appearance of disease symptoms represented by brown spot or the occurrence of necrosis and rot on them.

The pathogenicity of the fungal isolates (Trichoderma sp.) was performed as in bacteria, except for the pathological symptoms, that were represented by green rot, the occurrence of cracks and necrosis on the fruit bodies.

2.3 Preparation of plant extracts

The largest quantity of newly-grown plants used in the study was collected. Atriplex tatarica was collected from the main site of Anbar University, and Haloxylon salicornicum was collected from the Western Anbar Desert, 15-20 km away from the international highway in west of the city of Ramadi. A. tatarica was collected on 30/8/2020, while H. salicornicum was collected on 16/9/2020, then it was washed with tap water to remove the dust, then it was cut into small pieces 2-3 cm to facilitate drying and grinding. The pieces dried until its weight was stable, and after complete drying, it was ground in the electric mill until it became a fine powder. To prepare the alcoholic extract, 100 mL of ethanol (80%) was added to 20g of the powder of each plant then incubated in shaking incubator at 4 °C (120 rpm) for 24 hours, the extract was filtered using a three-layer gauze cloth, then filtered with Whatman No. 1 filter paper. The extract was concentrated in a Rotary evaporator at 40°C to remove the largest amount of solvent, then the concentrated extract was dried at 40°C and placed in sealed bottles. The aqueous extract was prepared at same steps except use 100mL of the sterile distilled water instead of the ethanol [11].

2.4 Detection of active ingredients

2.4.1 Total Phenols

The method reported by [12], was used for total phenols assay, total phenols estimated from the standard curve consisting of different concentrations of catechol versus the absorbance values at wavelength 515 nm for each concentration.

2.4.2 Glycosides

The method described by [13], was used for glycosides assay. Glycosides estimated from the standard curve consisting of different concentrations of securidaside versus the absorbance values for each concentration.

2.4.3 Saponins

Saponins were estimated by method of [14], where saponin determined by the standard curve consisting of different concentrations of saponin solution versus the absorbance values at 550 nm wavelength.

2.4.4 Tannins

The method described by [15], was used for determination of tannins. The standard curve consisting of different concentrations of tannic acid versus the absorbance values at a wavelength of 760 nm was used for tannins estimation.

2.4.5 Flavonoids

Flavonoids was estimated by method of [16], where flavonoids was estimated based on the standard curve consisting of different concentrations of rutin solution with absorbance values at 510nm for each concentration.

2.4.6 Total Alkaloids content (TAC)

It was estimated according to the method mentioned by [17], where total alkaloids estimated based on the standard curve consisting of different concentrations of atropine solution with absorbance values at a wavelength of 470 nm for each concentration.
2.5 Effectiveness of plant extracts against the pathogenic fungus T.harzianum

Concentrations of 1, 2, 3 and 4 mg/mL from each extract were prepared in the PDA media, then the dishes were inoculated with the pathogenic fungus (5 mm diameter) and after incubation at 25°C for 3 days, the diameters of the colonies were measured with a ruler [18].

2.6 Effectiveness of plant extracts against pathogenic bacteria P.tolaasii

The concentrations of the extracts were prepared as in the previous method, the bacteria were spread in the petri-dish (containing the Nutrient Agar medium) and 5 mm diameter wells were made in the middle of plate, 3 μl of each concentration was added to the well after incubation at 25°C for 24 hours, the area of the inhibition zone was measured by ruler [19].

2.7 Effect of the plant extracts on the fungus A. bisporus

The concentrations of the extracts were prepared as in the previous method. The concentrations of 1, 2, 3 and 4 mg/mL were prepared in the PDA medium, then the petri-dishes were inoculated with A. bisporus (5 mm diameter from a fresh colony-7 days old), after incubation at 25°C for 7 days, the colonies' diameters were measured with a ruler [20].

2.8 Effect of the plant powders on the pathogens and the fungi A.bisporus

The dry powder of both plants was used at a concentration of 0, 0.5, 1, 1.5 and 2% in the NA bacteria medium, then inoculated with 1 mL of the pathogenic bacteria suspension at a rate of 6.6 x 10^7 cells/mL. After the petri-dishes were incubated at 25°C for 24 hours, the bacterial colonies were counted. The effect of powders on the pathogenic fungus and edible mushroom A.bisporus, the same concentrations were used in the PDA medium, then the petridishes were inoculated with the two fungi separately, after incubation at 25°C for a period of (3 days for the pathogenic fungus) and (7 days for the fungus A.bisporus), the diameters of the colonies were measured with a ruler.

2.9 Statistical analysis

The analysis of variance was conducted using the SPSS program, and the comparison of means was conducted according to the Least Significant Deference (LSD) test at the probability level of 0.05 [21].

3. Results and Discussion

3.1 Isolation of P.tolassii isolates and assessment of their pathogenicity

Seven isolates of P. tolassii were isolated from the casing soil in the mushroom production farm, College of Agriculture - Tikrit University. Figure (1) shows the infection rate of A.bisporus fruit bodies with bacterial isolates, as this figure shows that the highest infection rate was recorded by isolate 1077, which had a rate of 77.45%, followed by isolate 1080 with an infection rate of 41.17%, and the lowest infection rate was recorded when infected in isolate 656, which amounted to 13.04%, while the remaining four isolates did not record any infection rate. The bacterial isolate 1077 was chosen as being the most pathogenic in the implementation of subsequent experiments.

![Figure 1](image.png)

**Figure 1.** Infection rates of of A.bisporus fruit bodies with Pseudomonas tolassii isolates.
3.2 Isolation of *T. harzianum* isolates and assessment of their pathogenicity

Eleven *T. harzianum* isolates were isolated from spent compost from Mushroom Farm, College of Agriculture - Tikrit University. According to the pathogenicity test, the results listed in Figure (2) showed that isolate 1076 showed the highest pathogenicity by recording the highest infection rate of 81.23%. The fungal isolate 1076 was chosen as being the most pathogenic in the implementation of subsequent experiments.

![Figure 2. Infection rates of *A. bisporus* fruit bodies with *T. harzianum* isolates.](image)

Through the infection rates of the *A. bisporus* fruit bodies with bacterial and fungal isolates as shown in Figures (1) and (2), some of *P. tolassii* and *T. harzianum* isolates caused disease in the fruit bodies at higher rates than the rest of the isolates may due to the physiological effectiveness and its enzymatic system that acts to degrade the tissues of the mushroom *A. bisporus*, or it may be reflected on the fungal and bacterial toxins that lead to damage and the emergence of pathological symptoms on the *A. bisporus* fruit bodies, and perhaps the reason for a difference in its pathogenicity is due to the different areas of the isolates collection with the different environmental conditions according to each geographical region, as well as the possibility of the presence of chemicals such as pesticides all these factors led to genetic variations among the isolates [22].

3.3 Determination of the active substances in *A. tatarica* and *H. salicornicum* extracts

Table (1) shows the active substances and their concentrations in the aqueous and alcoholic extracts of *A. tatarica* and *H. salicornicum*. The results of the total alkaloids showed significantly the superiority of the alcoholic extract of the *A. tatarica*, as it amounted to 35.14 mg/g extract, followed by the alcoholic extract of the *H. salicornicum*, which reached 31.07 mg/g extract, followed by the aqueous extract of the *A. tatarica* which amounted to 28.13 mg/g extract, while the lowest total alkaloids composition was for the aqueous extract of *H. salicornicum*, which amounted to 25.45 mg/g extract.

Tannins were significantly superior to the alcoholic extract of *A. tatarica*, which amounted to 44.79 mg/g extract, followed by the aqueous extract of same plant, which amounted to 31.52 mg/g extract, followed by the alcoholic extract of *H. salicornicum*, which amounted to 31.21 mg/g extract.

Saponins, which were also significantly superior to the alcoholic extract of *A. tatarica*, which amounted to 43.47 mg/g extract, followed by the alcoholic extract of *H. salicornicum*, which amounted to 36.27 mg/g extract, while the lowest saponins content was in the aqueous extract of *H. salicornicum*, which amounted to 31.21 mg/g extract.

Phenols was significantly superior to the alcoholic extract of *A. tatarica*, which amounted to 32.05 mg/g extract, followed by the alcoholic extract of *H. salicornicum*, which amounted to 17.62 mg/g extract, the lowest phenols content was in the aqueous extract of *H. salicornicum*, which amounted to 11.28 mg/g extract.

Alcoholic extract of *H. salicornicum* was significantly superior to the glycosides which amounted to 47.76 mg/g extract, followed by the aqueous extract of same plant, which amounted to 41.89 mg/g extract, while the lowest glycosides content was in *A. tatarica* aqueous extract, which was 32.25 mg/g extract.

Flavonoids were significantly superior to the alcoholic extract of *A. tatarica*, which amounted to 31.66 mg/g extract, followed by the alcoholic extract of *H. salicornicum*, which was 30.12 mg/g extract, the lowest flavonoids were in the *H. salicornicum* aqueous extract, which amounted to 21.14 mg/g extract.
Table 1. Active substances and their concentrations in the aqueous and alcoholic extracts of *A. tatarica* and *H. salicornicum*.

| Plant extracts                  | Active substances (mg/ g extract) | Flavonoids | Glycosides | Phenols | Saponins | Tannins | Alkaloids |
|---------------------------------|-----------------------------------|------------|------------|---------|----------|---------|-----------|
| Aqueous extract of *A. tatarica* | 23.0                              | 32.25      | 14.11      | 10.14   | 31.52    | 28.13   |
| Alcoholic extract of *A. tatarica* | 31.66                             | 36.19      | 32.05      | 43.47   | 44.79    | 35.14   |
| Aqueous extract of *H. salicornicum* | 21.14                             | 41.89      | 11.28      | 8.03    | 24.33    | 25.45   |
| Alcoholic extract of *H. salicornicum* | 30.12                             | 47.76      | 17.62      | 36.27   | 31.21    | 31.07   |
| L.S.D. 0.05                     | 1.53                              | 3.24       | 2.61       | 4.33    | 3.11     | 2.07    |

The results showed a variation in the concentration of the active substances in the studied plants as shown in Table (1), this may be due to the difference of each plant from the other at the level of species and the difference in genetic composition. It also differed on the level of solubility in the alcoholic and aqueous solvents. The reason for this is due to the solubility of the active substances in the alcohol is greater than that of its solubility in the water, as the above table shows the percentages of the active substances in the alcoholic extract is higher than that of the aqueous extract in all active compounds namely total alkaloids, tannins, saponins, total phenols, glycosides and flavonoids for both plants, this is agree with study of [23].

3.4 Effect of aqueous and alcoholic extracts of *A. tatarica* and *H. salicornicum* on the growth of pathogenic fungus *T. harzianum* (1076)

The results listed in Table (2) below showed the effect of aqueous and alcoholic extracts of *A. tatarica* and *H. salicornicum* on the growth of the pathogenic fungus *T. harzianum*. The minimum diameter of the *T. harzianum* colony for the average of the extracts was 6.03 cm in *A. tatarica* alcoholic extract, followed by the alcoholic extract of *H. salicornicum*, which reached 7.42 cm. For the concentration level, 4 mg / mL recorded the minimum diameter of *T. harzianum* colony which was 6.0 cm followed by the concentration 3 mg/mL, which reached 6.87 cm. While for the interaction level, the results show that the lowest diameter of the colony of *T. harzianum* was at a concentration of 4 mg / mL with *A. tatarica* alcoholic extract reached 3.10 cm, followed by the concentration 3 for the same treatment, which amounted to 4.43 cm. As shown in Figure (3), the control treatment and the alcoholic extract of *A. tatarica*, the latter gave the best result of inhibiting the pathogenic fungus *T. harzianum*.

Table 2. Effect of aqueous and alcoholic extracts of *A. tatarica* and *H. salicornicum* on the growth of pathogenic fungus *T. harzianum* (1076).

| Extract Type                               | Concentration (mg/mL) | Average of the extracts |
|--------------------------------------------|-----------------------|------------------------|
|                                            | 0  | 1  | 2  | 3  | 4  | T. harzianum colony diameter (cm) |
| Aqueous extract of *A. tatarica*           | 8.93 | 8.82 | 8.41 | 8.07 | 7.56 | 8.36 |
| Aqueous extract of *H. salicornicum*       | 9.0  | 8.93 | 8.66 | 8.46 | 8.21 | 8.65 |
| Alcoholic extract of *A. tatarica*         | 8.9  | 7.50 | 6.21 | 4.43 | 3.10 | 6.03 |
| Alcoholic extract of *H. salicornicum*     | 9.0  | 8.72 | 7.76 | 6.52 | 5.11 | 7.42 |
| Average of the concentrations              | 8.96 | 8.5  | 7.76 | 6.87 | 6.0  | Extract X |
| L.S.D. 0.05                                | 0.67 | Extract= 0.67 | Concentration= 0.71 | Concentration= 0.93 |

Figure 3. Effect of alcoholic extract of *A. tatarica* on the growth of the pathogenic fungus *T. harzianum* (left: with 4 mg/ml, Right: control).
3.5 Effect of aqueous and alcoholic extracts of A. tatarica and H. salicornicum on the growth of pathogenic bacteria P. tolaasii (1077).

The results listed in Table (3) showed the effect of water and alcoholic extracts of A. tatarica and H. salicornicum in inhibiting P. tolaasii bacteria. The highest diameter of the inhibition zone for the rate of the extracts was in the alcoholic extract of H. salicornicum which reached 8.52 mm, followed by (the alcoholic extract of A. tatarica) and (the aqueous extract of the H. salicornicum), which reached 1.11 mm, while at the level of concentration, the highest inhibition area was 5.29 cm for the concentration 4 mg / ml, followed by the concentration 3 mg / ml as the inhibition area reached 4.01 mm. For the interaction level, the results showed that the highest inhibition area was in the alcoholic extract of H. salicornicum at the concentration 4 mg/ml, which amounted to 15.67 mm, followed by the concentration of 3 mg/ml for the same treatment, which amounted to 11.56 mm, and as shown in Figure (4), the third and fourth concentrations of H. salicornicum alcoholic extract gave the highest inhibition rate against P.tolaasii.

Table 3. Effect of aqueous and alcoholic extracts of A. tatarica and H. salicornicum on the growth of pathogenic bacteria P. tolaasii (1077).

| Extract Type                      | Concentration (mg/mL) | Bacterial inhibition zone (mm) | Average of the extracts |
|-----------------------------------|------------------------|--------------------------------|------------------------|
| Aqueous extract of the A. tatarica| 0 0 0 1.2* 1.43        | 0.53                           |
| Aqueous extract of H. salicornicum| 0 0 1.27 1.92 2.37     | 1.11                           |
| Alcoholic extract of A. tatarica  | 0 1.16 1.31 1.37 1.70  | 1.11                           |
| Alcoholic extract of H. salicornicum| 0 6.63 8.74 11.56 15.67| 8.52                           |
| Average of the concentrations     | 0 1.95 2.83 4.01 5.29  |                                 |

L.S.D. 0.05 Extract= 0.81 Concentration= 0.96 Extract X 1.21

Figure 4. Effect of H. salicornicum alcoholic extract in inhibiting P. tolaasii growth.

The results showed a discrepancy in the effect of the alcoholic extract of A. tatarica and H. salicornicum compared with the aqueous extract against the pathogens used in the study, as shown in the tables (2) and (3), this may be due to the presence of the active substances represented by total alkaloids, tannins, saponins, total phenols, glycosides and flavonoids with higher concentrations and solubility in the alcoholic extract than in the aqueous extract, the effect of the alcoholic extract of A. tatarica against the fungus T. harzianum was higher than that of the aqueous extract of the same plant. The alcoholic extract of H. salicornicum against P. tolaasii was higher than the water extract of the same plant against this bacteria. Also these results could be because the presence of inhibitory compounds other than the active substances that were detected in this study in alcoholic extracts rather than aqueous extracts, such as the compound Apigenin, which was found in the extract of some plants, which has a role in reducing the mass of cell membranes and led to phenotypic changes, especially the shrinkage of cells and causing an increase in their permeability, leading to the entry of ions and sugars, subsequently, a defect in the physiological system was occur in the pathogens cells [24]. Perhaps the reason for the efficiency of alcoholic extracts of A. tatarica and H. salicornicum is different mechanisms of inhibition from what is in the aqueous extract, as there are several mechanisms of inhibition. The mechanism of inhibition may be mechanical, some of which affect the cell wall or proteins, or some extracts may act after drying as a solid membrane around the bacterial cells, fungal mycelium, and spores which affects their growth or affects the action of pathogenic enzymes [25,26]. The proportions of the active substances present in both plants differed and their effect on pathogens differed too, as the alcoholic extract was better than the aqueous extract in
inhibiting pathogens. The aqueous extract of *Lantana camara* was used against *Alternaria alternata*, which gave the lowest inhibition rate of 0.88%, while the methanolic extract was 50%, and when using ethanol and acetone extract, the percentage of inhibition was 100%. Also, the reason for the inhibition of pathogens by alcoholic extracts of *A. tatarica* and *H. salicornicum* or their raw powders may be due to the different inhibition mechanisms of the active substances in alcoholic extracts than it is in aqueous extracts.

The chemical and physical extract properties have also role in the pathogens inhibition effect, for example the mechanism of phenolic compounds act somewhat variable, some of which work to change the nature of proteins and disrupt the cell membranes functions by linking them to the active sites of cellular enzymes by OH groups that have the ability to form hydrogen bonds with those sites [27,28]. The mechanism of inhibition may be physical, as the adsorption of the extract (especially in pH 4-6.56) occurs on the surfaces of cells, spores and fungal hyphae, which affects the reproduction process. Thus the pathogenic growth can be inhibited [29].

Through the previous studies, it was found that the plant extracts contained tannins have an inhibitory effect on oxidative phosphorylation and prevent iron oxidation as well as having a role in inhibiting extracellular enzymes (Scalbert, 1991), and it was found that alkaloids lead to programmed death by breaking DNA bonds [30], [31], indicated that terpenes lead to an imbalance in the cytoplasm and increase the permeability of the membranes, as mentioned by [32], that terpenoids lead to the disintegration of the cell wall and an increase in permeability, while the role of glycosides in inhibiting bacteria and fungi, was very clear when dihydrodehydrodiconiferyl alcohol 9′-O-β-D-glucoside (DDDC9G) used with *Candida albicans* and caused affecting the pore size in the plasma membrane. Also it was observed that the cell volume decreased due to the changes caused by damage to the cell membranes when using DDDC9G [33].

The flavonoids and phenols extracted from *Duguetia furfuracea* inhibit the cytochrome enzyme P-450, which is involved in the synthesis of the fungal cell membrane, a number of which work by promoting damage to the function of the cell membrane or cell wall, and these phenolic compounds are Caffeic acid, Chlorogenic acid, Rutin, Quercitrin and Isoquercitrin [22,26]. On the other hand, saponines clearly affected the cell wall and an increase in the permeability of the some pathogenic membranes, the outer layer of the cell wall became thinner as for the middle layer, it increased in thickness and affected the cytoplasmic membrane and organelles such as the nucleus and mitochondria [34].

### 3.6 Effect of aqueous and alcoholic extracts of *A. tatarica* and *H. salicornicum* on the *A. bisporus* growth

The results listed in Table (4) showed the effect of aqueous and alcoholic extracts of *A. tatarica* and *H. salicornicum* on the *A. bisporus* growth, the lowest effect of the average of the extracts in the colony diameter of *A. bisporus* was in the alcoholic extract of *H. salicornicum*, which was 7.67 cm, followed by the aqueous extract of the *A. tatarica*, which was 7.74 cm. For the concentration average, the results showed that the lowest diameter of *A. bisporus* colony was at the concentration 4 mg / mL, which was 7.44 cm, followed by the concentration of 3 mg / mL, which was 7.66 cm , while in the interaction level, the results showed that the lowest diameter of the mushroom colony was in *H. salicornicum* alcoholic extract at a concentration of 4 mg / mL, which was 7.13 cm, followed by *A. tatarica* alcoholic extract of the same concentration, which was 7.21 cm.

#### Table 4. Effect of aqueous and alcoholic extracts of *A. tatarica* and *H. salicornicum* on the *A. bisporus* growth.

| Extract Type                  | Concentration (mg/mL) | Average of the extracts |
|-------------------------------|-----------------------|------------------------|
|                               | 0  | 1  | 2  | 3  | 4  |                      |
| Aqueous extract of the *A. tatarica* | 8.11 | 8.03 | 7.82 | 7.55 | 7.21 | 7.74 |
| Aqueous extract of *H. salicornicum* | 8.03 | 8.07 | 8.01 | 7.88 | 7.83 | 7.96 |
| Alcoholic extract of *A. tatarica* | 8.07 | 7.97 | 7.71 | 7.45 | 7.13 | 7.67 |
| Alcoholic extract of *H. salicornicum* | 8.10 | 8.0 | 7.93 | 7.77 | 7.59 | 7.88 |
| Average of the concentrations | 8.08 | 8.02 | 7.87 | 7.66 | 7.44 | 7.13 |
| L.S.D. 0.05 | Extract= 0.19 | Concentration= 0.22 | Extract X |

The results of the study also showed a discrepancy in the effect of aqueous and alcoholic extracts of both plants on the *A. bisporus* growth, as shown in Table (3), perhaps the reason for encouraging the growth of *A. bisporus* is that the alcoholic extracts helped fungal hyphae in absorbing nutrients, or may be increasing the concentrations of some substances in the alcoholic extract compared to the aqueous extract helped to raise the nitrogen content in the culture media, which led to a greater opportunity to benefit of *A. bisporus*, and this is consistent with [3]. *A. bisporus* classified as basidiomycetes fungus while *T. harzianum* belong to the ascomycetes fungus this differences may be explain the inhibitory effect in *T. harzianum* more than *A. bisporus*. 

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3.7 Effect A. tatarica and H. salicornicum powders on the growth of pathogenic fungus T. harzianum

The results listed in Table (5) showed the minimum diameter of the pathogenic fungus T. harzianum colony (in average of the powders) was in A. tatarica powder, of 7.34 cm, while at the level of concentrations, it was the lowest diameter of the colony of 6.04 cm at the concentration of 2.0%, followed by the concentration of 1.5%, as the diameter of the colony was 7.13 cm. For the level of interaction (Powders × Concentration), the minimum diameter of T. harzianum colony was 5.01 cm at the concentration of 2.0% of A. tatarica powder, followed by the concentration of 1.5% for the same treatment was 6.63 cm.

Table 5. Effect of A. tatarica and H. salicornicum powders on the T. harzianum growth.

| Powder Type | Concentration (%) | Average of the powders |
|-------------|-------------------|------------------------|
|             | 0     | 0.5  | 1     | 1.5   | 2     | T. harzianum colony diameter (cm) |
| A. tatarica | 9     | 8.86 | 7.21 | 6.63  | 5.01  | 7.34 |
| H. salicornicum | 9  | 9    | 8.66 | 7.63  | 7.06  | 8.27 |
| Average of the concentrations | 9     | 8.93 | 7.94 | 7.13  | 6.04  | 7.50 |
| L.S.D. 0.05 | Powders= 0.97 | Concentration= 1.16 | Powders X |

3.8 Effect A. tatarica and H. salicornicum powders on the growth of the bacteria P. tolaasii

The results listed in Table (6) showed the lowest bacterial number (for the average of the powders) was in H. salicornicum powder, which had 5.05 x 10^7 cells/mL, while (at the level of concentrations), the lowest bacterial account was at concentration of 2.0%, which had 4.17 x 10^7 cells/mL, followed by a concentration of 1.5%, which had 4.78 x 10^7 cells/mL, for the interference level, the lowest bacterial account was recorded at a concentration of 2.0%, which had 3.23 x 10^7 cells/mL for H. salicornicum powder, followed by 4.06 x 10^7 cells/mL for the same treatment at concentration of 1.5%.

Table 6. Effect of A. tatarica and H. salicornicum powders on the P. tolaasii growth.

| Powder Type | Concentration (%) | Average of the powders |
|-------------|-------------------|------------------------|
|             | 0     | 0.5  | 1     | 1.5   | 2     | Bacterial account (x 10^7) |
| A. tatarica | 6.6   | 6.53 | 6.16 | 5.5   | 5.1   | 5.98 |
| H. salicornicum | 6.6 | 6.32 | 5.03 | 4.06  | 3.23  | 5.05 |
| Average of the concentrations | 6.6   | 6.43 | 5.59 | 4.78  | 4.17  | 5.62 |
| L.S.D. 0.05 | Powders= 0.39 | Concentration= 0.77 | Powders X |

3.9 Effect A. tatarica and H. salicornicum powders on the growth of A. bisporus

The results listed in Table (7) showed that the highest diameter of A. bisporus colony (for the average powders) was in H. salicornicum powder, which was 8.65 cm, without significant differences with A. tatarica powder, while at the level of concentrations, the highest diameter of A. bisporus colony was recorded at the concentration of 2.0%, which was 9 cm, followed by the concentration of 1.5%, which was 8.85 cm. For the level of interaction (Powders × Concentration), the highest diameter of the mushroom colony was recorded at the concentration of 2.0% for both plants powder, which was 9 cm, followed by the H. salicornicum powder at concentration of 1.5%, which had 8.87cm, without significant differences with A. tatarica powder.

Table 7. Effect of A. tatarica and H. salicornicum powders on the A. bisporus growth.

| Powder Type | Concentration (%) | Average of the powders |
|-------------|-------------------|------------------------|
|             | 0     | 0.5  | 1     | 1.5   | 2     | A. bisporus colony diameter (cm) |
| A. tatarica | 8.3   | 8.35 | 8.61 | 8.83  | 9.0   | 8.62 |
| H. salicornicum | 8.3 | 8.43 | 8.66 | 8.87  | 9.0   | 8.65 |
| Average of the concentrations | 8.3   | 8.39 | 8.64 | 8.85  | 9     | 8.85 |
| L.S.D. 0.05 | Powders= 0.11 | Concentration= 0.27 | Powders X |
The study also showed the discrepancy in the use of *A. tatarica* and *H. salicornicum* powders compared to that of the aqueous and alcoholic extract in their effect on the pathogens used in the study. The results of the raw plants powder of *A. tatarica* and *H. salicornicum*, as shown in Table (5) and (6), showed that the plants powder had the highest effect on the pathogens, this may be due to the fact that the active substances found in the plant extracts differ from the substances found in the raw powder of both plants. In addition to the active substances, may contain other substances and compounds such as proteins, enzymes and mineral salts (had additional inhibitory effect), which led to a higher inhibition of pathogens. In case of edible mushroom *A. bisporus* ,the *A. tatarica* and *H. salicornicum* raw powders showed increase of colony diameter with increase of concentrations of both plants this may be due to present other compounds and nutrients that encourage the growth this mushrooms [28].

As a conclusion, it is possible to benefit from wild plants, such as *A. tatarica* and *H. salicornicum* powders in the field of supporting the growth of edible mushroom *A. bisporus*, in addition to the importance of their alcoholic extracts in inhibiting some fungal and bacterial pathogens as alternatives to chemical pesticides that negatively affect humans and their environment.

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