ISSN:1813-1646 (Print); 2664-0597 (Online)
*Tikrit Journal for Agricultural Sciences*
Journal Homepage: http://www.tjas.org
E-mail: tjas@tu.edu.iq

TJAS
Tikrit Journal for Agricultural Sciences

# Control of brown blotch disease caused by Pseudomonas tolaasii by some chemical and biological treatments and its effect on some productive traits of the edible mushroom Agaricus bisporus

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**KEY WORDS:**

A. bisporus, Pseudomonas tolaasii, P. fluva , biological control, Microelements, brown blotch disease

**ARTICLE HISTORY:**

**Received**: 31/05/2022
**Accepted**: 26/06/2022
**Available online:** 31/12/2022

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## ABSTRACT

The research was carried out in a mushroom farm, College of Agriculture, Tikrit University, five microelements were tested, namely, iron, manganese, molybdenum, Zinc and boron, and a combination of these five elements, in addition biological agent, Pseudomonas fluva, to control of the brown blotch disease caused by pathogenic bacteria Pseudomonas tolaasii on the edible mushroom Agaricus bisporus. The results showed that all the treatments of the micro-elements as well as the growth-promoting bacteria P. fluva led to a significant superiority in the higher productivity of A. bisporus fruit bodies compared to the treatment of the pathogenic bacteria P. tolaasii only. The highest productivity of the fruit bodies was reached in the treatment of the elements mixture with beneficial bacteria P. fluva, which amounted to 2675.6 g / 20 kg compost, with a biological efficiency of 79.59%. At the level of elements, molybdenum (without bacteria) and with P. fluva outperformed the other elements by recording the highest productivity of 2533.2 and 2527.4 g / 20 kg compost with a biological efficiency of 80.26 and 79.0% , respectively, compared to the lowest productivity of 1404.3 g / 20 kg compost with a biological efficiency of 45.7 in the pathogenic bacteria P. tolaasii. The results showed that all treatments of the elements individually and in combination led to a significant decrease in the infection rate, no infection rate was recorded in the treatments of the elements mixture with the presence of pathogenic bacteria P. tolaasii with beneficial P. fluva compared to the highest infection rate of 32% in the treatment of P. tolaasii.

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## INTRODUCTION

Several diseases infect farms of the edible mushroom *A. bisporus*, one of these diseases is brown blotch caused by the bacterium *Pseudomonas tolaasii* (Hassan , 2013; Hesse et al. 2018) . Tolaas in 1919 was the first to diagnose this disease worldwide, and it still poses a clear threat to mushroom

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farms around the world until the present time (Moore et al., 2006). This disease appears as small yellow blotches on the fruit bodies, which then expand with moisture and turn into brown and sometimes golden yellow (Cho and Kim, 2003; McGee et al., 2017), which reduces its marketing significance . The incidence of this disease reaches 41-60% of the total production, and sometimes the infection expands to the entire farm with high humidity and non-compliance with preventive measures (Vieira and Pecchia, 2017).

There are some mechanisms by which these bacteria invade the food mushroom farms *A. bisporus*. Some studies have indicated that the secretion of the toxin Tolassin, which is one of the most important virulent factors of these bacteria, while other studies have indicated that it produces various enzymes that break down the walls of the mushroom cell (Chung et al., 2014; Shun et al., 2018). These high rates of infection and their rapid spread require rapid and accurate treatment. Because pesticides and chemicals pose a challenge to mushroom producers, the productive life of the fruit body is very short, only 3-4 days, compared to vegetables and fruits, so it is very difficult to decompose these materials and pesticides (Lamichhane et al., 2018).

As a result of the pesticides dangers to human health and the environment, researchers have been turning to other methods such as safe chemical methods, biological methods, natural materials and physical means to limit the spread of this disease (Osdaghi et al., 2019), Studies have shown the presence of many bacterial species in the production environments of the mushroom *A.bisporus*, especially, the casing layer is the optimal place for the growth of this bacteria (Carrasco et al., 2019), different bacterial species capable of detoxifying tolasin are associated with edible fruit bodies and wild *Agaricus* spp. ( Tsukamoto et al., 2001). Noble et al., 2009: Zhang et al. 2016 reported that several *Pseudomonas* spp. encourage the growth of the *A.bisporus* by removing the intrinsic inhibitory compounds produced by the fungus itself, in addition, microelements have a structural importance in mushroom fruit bodies and may contribute to the *A.bisporus* against the diseases that infect it, (Stamets, 2005) showed that mushrooms contain several essential elements in their composition, and these elements are cofactors for many enzymatic reactions, such as molybdenum, zinc and manganese.

Due to the importance of the edible mushroom *A. bisporus* and the danger of infection with brown blotch disease that appears from time to time in mushroom production farms, the study aimed to evaluate the efficiency of the chemical treatment represented by the microelements in addition to the biological treatment, for control of brown blotch disease caused by *Pseudomonas tolaasii*.

## MATERIALS AND METHODS

The experiment was conducted in the Mushroom farm at the College of Agriculture - Tikrit University during the mushroom production cycle 22/11/2019 -22/1/2021, to study the effect of some chemical and biotic factors on the infection of *A. bisporus* with pathogenic bacteria *P. tolaasii*

### Cultivation of *A. bisporus*

Preparation of Mushroom breeding (*A. bisporus* / A15), compost, casing soil and all steps of producing fruit bodies of *A. bisporus* were carried out according to the standard method that was conducted in previous studies (Hassan et al., 2002; Hassan and Mahmoud, 2003; Hassan et al., 2022).

### Chemical treatments

Five micro-elements were selected: iron, manganese, molybdenum, zinc and boron. These elements were sprayed at a concentration of 100 ppm at 100 ml/m2 of casing soil while the sixth treatment included spraying 100 ml/m2 of a mixture of these elements in equal proportions.

### Biological treatments

Pathogenic bacteria, *P. tolaasii* and beneficial bacteria *P. fluva* were isolated from the casing layer of mushroom farm - Tikrit University - Iraq, which were molecularly characterized to the species level according to the nucleotide sequencing of 16 S rRNA gene (Aldarraji et al., 2022) the pathogenic bacteria *P.tolaasii* was added at concentration $1 \times 10^{10}$ bacterial cells/ml at a rate of $100 ml/m^2$ to the casing layer, after three days the casing layer was inoculated with the beneficial bacteria P. fluva at a concentration of $1 \times 10^{10}$ bacterial cells/ml at the rate of $100 ml/ m^2$ to the casing layer. Bacterial spraying was carried out on the experimental units weighing 18 kg compost with dimensions of 60 cm length, 30 cm width and 20 cm thickness.

**Experiment factors**

The experiment included 4 treatments. All teatments were carried out on the casing layer:

T1: Spraying with micro-elements without bacteria

T2 :Spraying with micro-elements + beneficial bacteria *P. fluva*

T3 : Spraying with micro-elements + pathogenic bacteria *P.tolaasii*

T4: Spraying with micro-elements + *P.tolaasii* + *P. fluva*

**Studied traits**

**productive traits**

The productivity of *A. bisporus* for the 1st flush was estimated by the weight of the fresh mushroom yield. The yield was calculated using the production formula per unit area (g fresh mushrooms / 20 kg of compost), then the biological efficiency was estimated, which expresses a relationship of fresh weight of the fruit bodies with the dry medium weight:

% Biological efficiency (B.E = fresh weight of the fruit bodies (kg) / dry weight of the medium (kg) x 100. (Beyer and Muthersbaugh 1996)

**Infection rate**

Infection rate was estimated as follow:

Infection rate = number of infected fruits ÷ total number of fruits x 100

The fruits were considered infected in the event of the appearance of pathological symptoms represented by brown-yellowish color and necrosis on the fruit bodies.

**Estimation of Dry weights**

Dry weights of fruiting bodies were determined 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, and 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**

**Effect of microelements, *P.tolaasii* and P. *fulva* on the weight of the *A. bisporus* yield**

Table (2) shows the effect of some micro-elements , pathogenic *P.tolaasii* and beneficial *P. fulva* bacteria and the interaction between them on the weight of the first flush of the *A. bisporus* fruit bodies. The treatment without both bacteria showed the highest yield for the weight of the first flush reached 2343.7 and 2331.1 g / 20 kg compost, compared to the lowest yield recorded in the treatment of pathogenic bacteria *P. tolaasii*, which reached 2003.8 g / 20 kg compost, with no significant differences between the means of all treatments. In the effect of micro-elements, zinc recorded the highest yield of the first flush, which amounted to 2442.1 gm / 20 kg compost, compared to the lowest yield, which was 1746.6 gm / 20 kg compost in the control, with no significant differences recorded between the means of the elements. At the level of interaction between bacteria and micro-elements, the highest productivity reached 2675.6 g / 20 kg compost in the treatment of the elements mixture with the beneficial bacteria, *P. fulva*, compared to the lowest yield recorded in the treatment of pathogenic bacteria without any element, which amounted to 1404.3 g / 20 kg compost.

**Table (1): Effect of microelements, *P.tola*asii and *P. fulva* on the weight of the first flush of *A. bisporus* yield (g/ 20 kg compost)**

| Treatments | Control | Fe | Mn | Mo | Zn | Bo | Mix. | Average of treatments |
|---|---|---|---|---|---|---|---|---|
| T1 | 2081.66 | 2332.05 | 2077.24 | 2533.22 | 2495.30 | 2403.58 | 2482.62 | 2343.70 |
| T2 | 2026.43 | 2222.82 | 2101.57 | 2527.43 | 2469.16 | 2300.75 | 2675.60 | 2331.12 |
| T3 | 1404.31 | 2075.97 | 1774.92 | 2180.73 | 2393.25 | 1970.07 | 2157.93 | 2003.80 |
| T4 | 1474.17 | 2376.10 | 1806.52 | 2251.68 | 2410.63 | 2124.43 | 2230.95 | 2086.34 |
| Average of micro-elements | 1746.66 | 2251.74 | 1940.09 | 2371.95 | 2442.11 | 2199.77 | 2386.82 | |
| LSD $_{0.05}$ | Micro-elements= 8.71 Treatments = 6.58 Micro-elements × Treatments= 17.42 | | | | | | | |

T1: Spraying with micro-elements without bacteria, T2 :Spraying with micro-elements + beneficial bacteria *P. fluva*, T3 : Spraying with micro-elements + pathogenic bacteria *P.tolaasii*, T4: Spraying with micro-elements + *P.tolaasii* + *P. fluva*

**Effect of microelements, *P.tolaasii* and *P. fulva* on the** biological efficiency (%) **of the *A. bisporus***

Table (3) shows the effect of some microelements and both bacteria *P.tolaasii* and *P. fulva* with the interaction between them on the biological efficiency of *A. bisporus* . The results of Table 2 showed the superiority of the beneficial bacteria *P. fulva* treatment in the biological efficiency, which reached to 71.362%, followed by 70.85% in the control. In the effect of the elements, the Zinc treatment showed a significant increase in the biological efficiency, which was 75.33%, while the lowest level of biological efficiency was 55.15% in the control. For the interaction effect between the two factors, bacteria and elements, the element mixture with *P. fulva* was superior on other treatment resulting in 79.59%, compared to the control treatment without elements, which reached to 65.14%.

**Table (2): Effect of microelements, *P.tolaasii* and *P. fulva* on the biological efficiency (%) of the *A. bisporus* yield**

| Treatments | Control | Fe | Mn | Mo | Zn | Bo | Mix. | Average of treatments |
|---|---|---|---|---|---|---|---|---|
| T1 | 63.76 | 71.82 | 61.46 | 80.26 | 79.85 | 65.48 | 73.35 | 70.85 |
| T2 | 65.14 | 68.87 | 61.17 | 79.00 | 78.99 | 66.76 | 79.59 | 71.36 |
| T3 | 45.70 | 61.72 | 54.25 | 68.18 | 69.96 | 57.80 | 67.68 | 60.75 |
| T4 | 45.99 | 68.29 | 53.07 | 69.12 | 72.52 | 60.04 | 65.85 | 62.12 |
| Average of micro-elements | 55.15 | 67.67 | 57.48 | 74.14 | 75.33 | 62.52 | 71.61 | |
| LSD $_{0.05}$ | Micro-elements= 0.28 Treatments=0.21 Micro-elements × Treatments= 0.56 | | | | | | | |

T1: Spraying with micro-elements without bacteria, T2 :Spraying with micro-elements + beneficial bacteria *P. fluva*, T3 : Spraying with micro-elements + pathogenic bacteria *P.tolaasii*, T4: Spraying with micro-elements + *P.tolaasii* + *P. fluva*

**Effect of microelements, *P.tolaasii* and *P. fulva* on the dry weight of the *A. bisporus***

Table (4) shows the effect of some micro-elements , pathogenic *P.tolaasii* and beneficial *P. fulva* bacteria and the interaction between them on the percentage of the first flush of *A. bisporus* dry weight. The highest dry weight was 9.72% followed by 9.51%, in micro-elements (without bacteria) treatment and with beneficial *P. fulva*, respectively, while the lowest dry weight 9.22% was recorded in present of pathogenic *P.tolaasii* . In the effect of microelements on the dry weight, the treatment of the elements mixture was 9.95% compared to the lowest dry weight of 8.87% in the control

The same table shows the interaction between the two factors on the percentage of fruit body dry weight ,the results showed the highest dry weight was 10.43% followed by 10%, in micro-elements (without bacteria) treatment and with both bacteria, respectively,

**Table (3): Effect of microelements, *P.tolaasii* and *P. fulva* on the dry weight (%) of the *A. bisporus***

| Treatments | Control | Fe | Mn | Mo | Zn | Bo | Mix. | Average of treatments |
|---|---|---|---|---|---|---|---|---|
| T1 | 9.56 | 10.16 | 9.16 | 9.35 | 9.90 | 9.47 | 10.43 | 9.72 |
| T2 | 9.60 | 9.43 | 9.28 | 9.42 | 9.70 | 9.35 | 9.80 | 9.51 |
| T3 | 8.18 | 9.36 | 9.11 | 9.28 | 9.60 | 9.31 | 9.72 | 9.22 |
| T4 | 8.14 | 9.39 | 8.91 | 9.48 | 9.61 | 9.25 | 10.00 | 9.25 |
| Average of micro-elements | 8.87 | 9.58 | 9.11 | 9.38 | 9.70 | 9.34 | 9.95 | |
| LSD $_{0.05}$ | Micro-elements= 0.02 Treatments= 0.015 Micro-elements × Treatments=0.04 | | | | | | | |

T1: Spraying with micro-elements without bacteria, T2 :Spraying with micro-elements + beneficial bacteria *P. fluva*, T3 : Spraying with micro-elements + pathogenic bacteria *P.tolaasii*, T4: Spraying with micro-elements + *P.tolaasii* + *P. fluva*

**Effect of microelements, *P.tolaasii* and *P. fulva* on the infection rate of the *A. bisporus***

The results showed that all treatments of the micro-elements individually and micro-elements with both bacteria led to a significant decrease in the infection rate, no infection rate was recorded in the treatments of the micro-elements mixture with the presence of pathogenic bacteria *P. tolaasii* and the pathogenic *P. tolaasii* with beneficial *P. fluva* compared to the highest infection rate of 32% in the treatment of *P. tolaasii.*

**Table (4): Effect of microelements, *P.tolaasii* and *P. fulva* on the infection rate (%) of the *A. bisporus***

| Treatments | Control | Fe | Mn | Mo | Zn | Bo | Mix. | Average of treatments |
|---|---|---|---|---|---|---|---|---|
| T1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| T2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| T3 | 32.13 | 1.35 | 8.19 | 0.00 | 1.36 | 0.90 | 0.00 | 6.85 |
| T4 | 28.81 | 1.28 | 7.98 | 1.59 | 1.71 | 4.17 | 0.00 | 6.79 |
| Average of micro-elements | 14.98 | 0.66 | 4.04 | 0.40 | 0.77 | 1.27 | 0.00 | |
| LSD $_{0.05}$ | Micro-elements= 1.008 Treatments= 0.76 Micro-elements × Treatments=2.017 | | | | | | | |

T1: Spraying with micro-elements without bacteria, T2 :Spraying with micro-elements + beneficial bacteria *P. fluva*, T3 : Spraying with micro-elements + pathogenic bacteria *P.tolaasii*, T4: Spraying with micro-elements + *P.tolaasii* + *P. fluva*

Through the results of the experiment, it is clear that the micro-elements have an effect on the pathogenic bacteria, which is reflected on the amount of mushroom yield production in a direct and indirect way, these results are close with a study of Szolnoki and Farsang (2013). This effect may be due to the fact that these elements block the sites of influence in the enzymes of pathogenic bacteria, which leads to the displacement of some cations necessary for the functioning of the cell in the organism, which inhibits the action of the enzymes and toxins of pathogenic bacteria. On the other hand, the fruiting bodies of *A. bisporus* need these elements for their growth and increase in fruit bodies sizes and to improve the level of life activities such as absorption and respiration

(Hassan et al., 2017). In addition, the presence of these elements may play the role of encouraging the developing fungal hyphae to shift from the vegetative phase to the formation of pinheads, other reason, the elements here play an important role in the healing of wounds and necrosis caused by pathogenic bacteria secretions, and also may play a role in reducing the number of bacteria scattered in the medium by increasing the osmotic pressure, which works to decomposition of bacterial cell membranes.

The results showed that zinc and molybdenum outperformed in reducing the infection of pathogenic bacteria, these results agreed to some extent with the findings of (Cho and Kim, 2003), as it was shown that zinc has a high ability to prevent the toxin of pathogenic bacteria tolasin from forming channels that permeate the cell membranes of the fungus *A. bisporus*. Thus, it loses its selective ability to permeability and ion exchange, Thus, it prevents the leakage of the contents of the cytoplasm. Geunhyeong (2011) demonstrated that ion-induced channels by tolassin toxin can be inhibited by zinc through zinc penetration of the lactone ring, thereby inhibiting the action of tolassin toxin. The explanation for this disparity between the effect of microelements may be due to the different roles of these elements in living cells.

In terms of *P. fluva* effect in reducing infection and encouraging the growth of *A. bispor*us, these results are in agreement with study of Osdaghi et al. (2019) , which found that some species of bacteria not only protect the mushroom *A. bisporus* from pathogens, but also promote the growth of fruit bodies, while other species indirectly increase production by protecting them from pathogens, the reason for this may be due to several factors, including competition for food and air sites, or perhaps these bacteria secrete substances that may be enzymatic or other that degrade the pathogenic bacteria walls, other reason, perhaps the effect is not on bacteria as much as it deals with their secretions such as tolassin or decomposing enzymes, thus inhibiting their work or denaturant their structure. The enzymes secreted by *P.tolaasii* may face several inactivation factors, such as the concentration of the substance in the reaction substrate or temperature. The optimum temperature to increase the speed of enzyme activity is between 27-40, so enzyme activity may weaken when the temperature for fruiting is reduced to 12- 16$^{o}$C (Hassan et al., 2022).

Finally, the beneficial bacteria may secrete substances that inhibit the action of the pathogenic bacteria enzymes by competing for the substrate sites of the surface of the enzyme, or by secreting non-competitive inhibitors through a defect in the active group of the enzyme.

## Conclusion

In order to control the bacterial blotch disease caused by *P.tolaasii* bacteria on the fruit bodies of *A. bisporus*, the current study proved the importance of microelements, especially zinc and molybdenum, as well as the growth-promoting bacteria *P. fluva* in reducing infection with this disease and increasing the productivity of *A. bisporus*, these treatments are considered as an effective, cheap and safe alternative to chemicals.

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## السيطرة على مرض التبقع البني المتسبب عن Pseudomonas tolaasii بواسطة بعض المعاملات الكيميائية والاحيائية وتأثيرها على بعض الصفات الإنتاجية للفطر الغذائي Agaricus bisporus

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**الكلمات المفتاحية:**
A. bisporus، Pseudomonas tolaasii، Pseudomonas fluva، المقاومة الاحيائية ، العناصر الصغرى ، مرض التبقع البني.

**الخلاصة**

أجري البحث في مزرعة فطر، كلية الزراعة- جامعة تكريت، تم اختبار خمسة عناصر صغرى وهي الحديد و المنغنيز و المولبيدينوم و الزنك والبورون وتوليفة من هذه العناصر الخمسة ، فضلا عن العامل الحيوي، بكتريا Pseudomonas fluva لمقاومة مرض التبقع البني التي تسببها البكتيريا Pseudomonas tolaasii على الفطر الصالح الغذائي Agaricus bisporus. أظهرت النتائج أن جميع معاملات العناصر الصغرى وكذلك بكتيريا P. fluva المعززة للنمو أدت إلى تفوق معنوي في زيادة إنتاجية ألاجسام الثمرية للفطر A. bisporus مقارنة بمعاملة البكتيريا الممرضة P. tolaasii فقط. بلغت أعلى إنتاجية في معاملة خليط العناصر مع بكتيريا P. fluva اذ بلغت 2675.6 غم / 20 كغم سماد وبكفاءة بيولوجية 79.59٪. على مستوى العناصر ، تفوق الموليبدينوم (بدون بكتريا) وبوجود بكتيريا P. fluva على العناصر الأخرى بتسجيل أعلى إنتاجية قدرها 2533.2 و 2527.4 غم / 20 كغم سماد وبكفاءة بيولوجية 80.26 و 79.0٪ على التوالي ، مقارنة بأدنى إنتاجية 1404.3 غم / 20 كجم سماد ، بكفاءة بيولوجية 45.7 في البكتيريا الممرضة P. tolaasii. أظهرت النتائج ايضا أن جميع معاملات العناصر منفردة ومجتمعة أدت إلى انخفاض معنوي في معدل الإصابة ، ولم يتم تسجيل اي إصابة في معاملات خليط العناصر مع البكتيريا الممرضة P. tolaasii وبوجود بكتيريا P. fluva المفيدة مقارنة بأعلى معدل إصابة بنسبة 32٪ في معاملة البكتريا الممرضة P. tolaasii فقط.