Estimating the Effectiveness of some biological agents and Palm biochar in control of Dry bubble disease on the second flush of White mushroom Agaricus bisporus

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ABSTRACT
In a time when technology is present in every aspect of our lives, it is crucial to incorporate advanced solutions to protect sensitive medical data in Social Medical Systems (SMS). This study explores the need to improve security in public healthcare by using advanced technologies to strengthen the weaknesses in the growing field of Social Medical Systems. This study specifically examines the analysis of IoT-23 data using machine learning (ML) and deep learning (DL) methods, as technology and healthcare converge. The research highlights the increasing significance of technology in healthcare, specifically focusing on the revolutionary emergence of Social Medical Systems. As these interlinked networks reshape the provision of public healthcare services, security challenges such as data breaches, cyber threats, and privacy concerns become crucial barriers that require innovative solutions. The study utilizes a wide range of machine learning (ML) and deep learning (DL) techniques to examine IoT-23 data, offering a detailed comprehension of the security environment in Social Medical Systems. The chosen models comprise Support Vector Machines (SVM), Isolation Forest, Random Forest, Convolutional Neural Networks (CNN), and Autoencoder. The results and discussions focus on evaluating metrics such as accuracy, precision, recall, and F1 score. These metrics provide insights into how effective each model is in identifying vulnerabilities.

1. Introduction

The study was conducted in the Plant Pathology Laboratory and the Mushroom Farm of Plant Protection Department at the College of Agriculture/Tikrit University/Iraq. The study aimed to evaluate the effectiveness of the bacteria *Paeniglutamicibacter psychrophenolicus* strain IRAQ-11, the yeast *Metschnikowia pulcherrima* strain IRAQ-14, and palm biochar in controlling of dry bubble disease on the mushroom *Agaricus bisporus* that caused by fungus *Lecanicillium fungicola* for the second flush. The (bacteria + palm biochar + pathogenic fungus) treatment showed the best results in control of the pathogenic fungus, as the infection rate reached 19.18%, followed by the (palm biochar + pathogenic fungus) treatment, with an infection rate of 24.19%, compared to the (pathogenic fungus only) treatment, which amounted to 73.39%. While the lowest severity of infection was 18.96% in the (bacteria + palm biochar + pathogenic fungus) treatment, followed by the (palm biochar + pathogenic fungus) treatment, with an severity of infection 20.32%, compared to the highest severity of infection in the (pathogenic fungus only) treatment, which amounted to 71.92%. The same treatments gave the best results in terms of the fresh weight of the fruiting bodies. The highest fresh weight of the fruiting bodies reached 1429.28 g in the treatment (bacteria + palm biochar + pathogenic mushrooms), followed by the treatment (palm biochar + pathogenic mushrooms) with a weight of 1351.5 g. compared to the lowest weight in Treatment (pathogenic fungi only), which amounted to 688.57 g. The highest rate of hardness of fruiting bodies was 8.09 kg/cm^2 in the treatment (bacteria + palm biochar + pathogenic fungi), followed by the treatment (palm biochar + pathogenic fungus) which amounted to 7.27 kg/cm^2, compared to the lowest rate. Hardness in the treatment (pathogenic fungi only) amounted to 2.79 kg/cm^2.

The White button mushroom, *Agaricus bisporus*, is most widely cultivated species of edible mushroom. It occupies the remaining 15% of the global production of mushrooms (Royse et al., 2017) due to the nutritional and health benefits it contains (Usman et al., 2021). As it represents sugars, proteins, enzymes, amino acids, phenols, carbohydrates and Peptides are bioactive compounds derived from mushrooms that are beneficial to human health and have antimicrobial activity, including RNA and DNA viruses (Seo and Choi, 2021). This activity has been used to treat

microorganisms pathogenic to humans (Podkowa et al., 2021) and plant pathogens (Saadi and Hassan, 2023).

A. bisporus is exposed to many diseases that are found in the quantity and quality of mushroom products, such as fungal, bacterial, and viral diseases (Altaf et al., 2022). Among the most important common fungal diseases affecting A. bisporus is dry bubble disease caused by the pathogen *Lecanicillium fungicola* (Amin et al., 2021), which is one of the most threatening fungal diseases to cultivated mushrooms worldwide and one of the biggest problems in the commercial production of the white edible mushroom *A. bisporus*. (Rokni et al., 2020). What increases the difficulty of controlling this disease is the availability of a suitable environment for increasing the pathogenic inoculum, in addition to the fact that the pathogen is also a fungus (Todorović et al., 2012).

Biochar produced from oak increased bacterial diversity in the root zone, activating plant defenses and suppressing the pathogenic fungus *Botrytis cinerea*, which causes gray mold on strawberries (De Tender et al., 2016). But there is still a gap in studies about biochar direct effect on pathogenic fungi. Bacteria and yeasts also work as biological control agents to control pathogens. Luo et al. (2019) reported that the bacteria *P. psychrophenolicus* inhibited the fungus *Alternaria alternate* by 70%, while the yeast *Metschnikowia pulcherrima* inhibited the filamentous growth of the fungus *Botrytis cinerea* by 79% (Millan et al., 2022).

2. Materials and methods

Pathogenic fungus

A highly pathogenic isolate was obtained from the mushroom production farm laboratories at the College of Agriculture/Tikrit University, molecularly diagnosed and registered with NCBI under the accession number OR554115.1.

Cultivation of the fungus A. bisporus and production of its fruiting bodies

The Compost medium was prepared according to (Hassan et al., 2002). The process of inoculating the compost medium at a rate of 2% was carried out by mixing the inoculum with it in polyethylene bags (length 60 cm, width 37 cm, height 20 cm). The bags were incubated in the growth hall at 25 degrees Celsius. After the mycelium had completed growing and spreading through the medium, the bags were covered with a 3 cm layer of peat moss, raising the humidity of the room to 85% to prevent dryness, and maintaining the temperature at 25 degrees Celsius for a week. After growth was complete and buds began to form, the temperature was reduced to about 16-18 degrees celsius using cooled outside air, and with the formation of mycelium tissue masses, the humidity was raised to 90%. When the beginnings of the fruiting bodies appeared, they were sprayed with water mist using sprinklers with fine spray holes. The harvesting process was carried out after 14-16 days from the covering time after reducing the relative humidity to 88%. The CO2 ratio was set at 1100 - 1300 parts per million and the temperature was maintained at around 18 degrees celsius.. The period between one flush and another was 7-10 days (Hassan et al. 2022).

Treatment with bio-palm charcoal, bacteria and yeast

Palm Biochar was added to the casing layer at a rate of 5%, mixed well with the casing layer based on laboratory trial results. The experimental units were sprayed with 100 ml of the suspension of the pathogenic fungus *L. fungicola* at 10^8 cfu (colony-forming units)/ml, then were covered with the nylon and left for a 3-day incubation period under cultivation room conditions before conducting the experimental treatments. For bacterial and yeast treatments, 100 ml of the cell suspension was sprayed after 3 days of treating with the pathogenic fungus at 10^8 cfu/ml. These treatments were applied to the white strain (A15) and the brown mushroom strain obtained from Mushroom farm – College of Agriculture/ University of Tikrit, Iraq.

Infection rate

It was measured as follows:

Infection rate = number of infected fruiting bodies ÷ total number of fruiting bodies × 100

Severity of Infection

The severity of infection was measured based on the pathological evidence that suggested in this study : 0 = healthy fruit bodies, 1 = small brown necrotic spots at a rate of 20%, 2 = necrotic spots at a rate of 50%, 3 = complete discoloration of the entire fruit body with cracking, 4 = decay and deformation of the fruit body. The severity of infection was calculated according to the equation by McKinney (1923) as follows:

Infection severity =

$$\frac{\boldsymbol{Sum(The\ number\ of\ infected\ plants\ at\ degree\ 0 * 0 + \ldots\ldots + the\ number\ of\ infected\ plants\ at\ degree\ 4 * 4)}}{\boldsymbol{Total\ number\ of\ plants * highest\ score\ in\ the pathological\ evidence}} \times \mathbf{100}$$

Fresh mushroom yield weight

The fruit bodies were weighed in grams per 20 kg of compost using a scale.

Fruit body hardness

Hardness in kg/ cm^2 was measured using a hardness measurement device.

Statistical analysis

The experiments of this study were carried out in a completely randomized design (CRD). An analysis of variance was carried out using the SPSS program, and the averages were compared according to the Least Significant Deference (LSD) test at a probability level of 0.05 (Al-Rawi and Khalaf Allah, 1980).

3. Results and Discussion

Infection rate

Table (1) indicates the effectiveness of the bacteria *P. psychrophenolicus* IRAQ-11, the yeast *M. pulcherrima* IRAQ-14, and palm biochar and their effect on the infection rate of two strains of the edible mushroom *A. bisporus* (white and brown) infected with the pathogenic fungus *L. fungicola* IRAQ-13 for the second flush. The highest effect on the treatment level was reached in the (bacteria + biochar + pathogenic fungus) treatment with an infection rate of 19.18%, followed by the (biochar + pathogenic fungus) treatment with an infection rate of 24.19%, compared to the highest infection rate in the (pathogenic fungus only) treatment, which amounted to 73.39. %, as for the level of fungal strains, the highest effect was reached in the brown strain with an infection rate of 23.82% compared to the lowest effect in the white strain with an infection rate of 26.69%. As for the interaction level, the treatment (bacteria + biochar + pathogenic fungus) was given to the brown strain. The highest effect was with an infection rate of 17.11%, compared to the lowest effect in the treatment (pathogenic fungi only) in the white strain, with an infection rate of 75.25%.

Table (1) The effect of the bacteria *P. psychrophenolicus* IRAQ-11, the yeast *M. pulcherrima* IRAQ-14, and palm biochar on the infection rate of two strains of the fungus *A. bisporus* with the pathogenic fungus *L. fungicola* IRAQ-13 for the second flush.

Treatments	*A. bisporus* strains		Average of Treatments
	White strain A15	Brawn strain	
Control	0	0	0
Pathogenic fungus *L. fungicola*	75.25	71.52	73.39
Palm biochar	0	0	0

Palm biochar + *L. fungicola*	25.65	22.72	24.19
P. psychrophenolicus + *L. fungicola*	27.70	25.19	26.45
P. psychrophenolicus + Palm biochar + *L. fungicola*	21.25	17.11	19.18
M. pulcherrima + *L. fungicola*	33.5	30.20	31.85
M. pulcherrima + Palm biochar + *L. fungicola*	30.15	28.28	30.15
Average of strains	26.69	23.82	
LSD 0.05	Treatments; 2.04 Strains; 2.15Treatments× strains; 3.50		

Severity of infection

The results listed in Table (2) indicate the effectiveness of the bacteria *P. psychrophenolicus* IRAQ-11, the yeast *M. pulcherrima* IRAQ-14, and palm biochar and their effect on the severity of infection of two strains of the edible mushroom *A. bisporus* (white and brown) infected by the pathogenic fungus *L. fungicola* IRAQ- 13 for the second flush, as the highest effect on the level of treatments was reached in the (bacteria + biochar + pathogenic fungus) treatment, with an infection severity of 18.96%, followed by the (biochar + pathogenic fungus) treatment, with an infection severity of 20.32%, compared to the highest infection severity in the (pathogenic fungus only) treatment amounting to 71.92%, while at the level of fungal strains, the highest effect was reached in the brown strain with an infection severity of 22.28%, compared to the lowest effect in the white strain with an infection severity of 23.68%. As for the interaction level, the treatment (bacteria + biochar + pathogenic fungus) of the brown strain had the highest effect with an infection severity of 18.13%, compared to the lowest effect in the (pathogenic fungus only) treatment in the white strain with an infection severity of 74.81%.

Table (2) The effect of the bacteria *P. psychrophenolicus* IRAQ-11, the yeast *M. pulcherrima* IRAQ-14, and palm biochar on the severity of infection of two strains of the mushroom *A. bisporus* with the pathogenic fungus *L. fungicola* IRAQ-13 for the second flush.

Treatments	***A. bisporus* strains**		**Average of Treatments**
	White strain A15	**Brawn strain**	
Control	0	0	0
Pathogenic fungus *L. fungicola*	74.81	69.02	71.92
Palm biochar	0	0	0
Palm biochar + *L. fungicola*	21.02	19.62	20.32
P. psychrophenolicus + *L. fungicola*	22.76	21.94	22.35
P. psychrophenolicus + Palm biochar + *L. fungicola*	19.78	18.13	18.96
M. pulcherrima + *L. fungicola*	26.44	25.71	26.08
M. pulcherrima + Palm biochar + *L. fungicola*	24.63	23.82	24.23
Average of strains	23.68	22.28	
LSD 0.05	Treatments; 1.86 Strains; 1.71 Treatments× strains; 2.23		

fresh weight

Table (3) shows the effect of the bacteria *P. psychrophenolicus* IRAQ-11, the yeast *M. pulcherrima* IRAQ-14, and palm biochar on the fresh weight of two strains of the edible mushroom *A. bisporus* (white and brown) infected with the pathogenic fungus *L. fungicola* IRAQ-13 for the second flush, as The highest effect was reached at the treatment level in the treatment (bacteria + biochar +

pathogenic fungi) with a weight of 1429.28 g., followed by the treatment (biochar + pathogenic fungi) with a weight of 1351.5 g., compared to the lowest weight in the treatment (pathogenic fungi only) which amounted to 688.57 g. As for the strains level, The fungus had the highest effect on the brown strain, weighing 1414.39 g., compared to the lowest effect on the white strain, weighing 1053.2 g. As for the level of interaction, the treatment (bacteria + biochar + pathogenic mushrooms) of the brown strain gave the highest effect, weighing 1673.22 g., compared to the lowest effect in Treatment (pathogenic fungi only) in the white strain, weighing 646.11 g.

Table (3) Effect of the bacteria *P. psychrophenolicus* IRAQ-11, the yeast *M. pulcherrima* IRAQ-14, and palm biochar on the fresh weight of two strains of the mushroom *A. bisporus* infected with the pathogenic fungus *L. fungicola* IRAQ-13 for the second flush.

Treatments	***A. bisporus* strains**		**Average of Treatments**
	White strain A15	**Brawn strain**	
Control	1117.50	1521.12	1319.31
Pathogenic fungus *L. fungicola*	646.11	731.03	688.57
Palm biochar	1159.37	1688.25	1423.81
Palm biochar + *L. fungicola*	1148.32	1554.67	1351.50
P. psychrophenolicus + *L. fungicola*	1103.07	1443.67	1273.37
P. psychrophenolicus + Palm biochar + *L. fungicola*	1185.34	1673.22	1429.28
M. pulcherrima + *L. fungicola*	1018.16	1306.04	1162.1
M. pulcherrima + Palm biochar + *L. fungicola*	1047.67	1397.12	1222.40
Average of strains	1053.20	1414.39	
LSD 0.05	Treatments; 132.9 Strains; 223.5 Treatments× strains; 321.3		

Fruit bodies hardness

Table (4) shows the effect of the bacteria *P. psychrophenolicus* IRAQ-11, the yeast *M. pulcherrima* IRAQ-14, and palm biochar on the hardness rate of the fruiting bodies of two strains of the edible mushroom *A. bisporus* (white and brown) infected with the pathogenic fungus *L. fungicola* IRAQ-13 for the second flush. The highest effect on the treatment level was reached in the (bacteria + biochar + pathogenic fungus) treatment with a hardness rate of 8.09 kg/cm^2, followed by the (biochar + pathogenic fungus) treatment with a hardness rate of 7.27 kg/cm^2 compared to the lowest hardness rate in the (pathogenic fungus only) treatment amounting to 2.79 kg/cm^2. At the level of mushroom strains, the highest effect was reached in the white strain with a hardness rate of 7.32 kg/cm^2 compared to the lowest effect in the brown strain with a hardness rate of 7.1 kg/cm^2. As for the intervention level, the treatment (bacteria + palm biochar + pathogenic fungi) of The white strain had the highest effect with a hardness rate of 8.42 kg/cm^2, compared to the lowest effect in the treatment (pathogenic fungi only) in the brown strain with a hardness rate of 2.75 kg/cm^2.

Table (4) The effect of the bacteria *P. psychrophenolicus* IRAQ-11, the yeast *M. pulcherrima* IRAQ-14, and palm biochar on the hardness rate of the fruiting bodies of two strains of the mushroom *A. bisporus* infected with the pathogenic fungus *L. fungicola* IRAQ-13 for the second flush.

Treatments	***A. bisporus* strains**		**Average of Treatments**
	White strain A15	**Brawn strain**	
Control	9.3	9	9.15
Pathogenic fungus *L. fungicola*	2.82	2.75	2.79
Palm biochar	10.1	10	10.05
Palm biochar + *L. fungicola*	7.37	7.16	7.27

P. psychrophenolicus + *L. fungicola*	7.23	7.11	7.17
P. psychrophenolicus + Palm biochar + *L. fungicola*	8.42	7.76	8.09
M. pulcherrima + *L. fungicola*	6.51	6.42	6.47
M. pulcherrima + Palm biochar + *L. fungicola*	6.78	6.57	6.68
Average of strains	7.32	7.10	
LSD 0.05	Treatments; 0.74 Strains; 0.17 Treatments × strains; 0.88		

The results showed that all treatments reduced the rate and severity of infection Unevenly, as the inhibitory effectiveness of yeasts and bacteria can be attributed to their role in competing for nutrients and the growth environment and producing volatile organic compounds and alcohols that inhibit the filamentous growth of Ascomycetes fungi, as well as suppressing and preventing the germination of conidial spores (Chen et al., 2018; Oro et al., 2018; Choińska et al., 2020; Piasecka-jo and Choin, 2020); Hydrolytic enzymes degrade cell walls and break down polysaccharides or reduce their level in the cell wall of pathogenic fungi (Maluleke et al., 2022). Also, the *P. psychrophenolicus* bacteria secretes a cyclic tetrapeptide called Arthropeptide {cyclo-(L-Pro-L-Leu-L-cHyp-L-Tyr)} which has antifungal activity, especially Ascomycetes, as it works to disrupt fungal cell membranes, tear apart their walls, and inhibit Germination of pathogenic fungal spores, which prevents its growth (Ramlawi et al., 2021; Ramlawi et al., 2022; Gomez et al., 2023). In addition to the interaction between bacteria and edible mushroom, which works to enhance the vegetative and reproductive growth of the fungus, enhance the substrate with nutrients, and improve the mushroom productivity (Frey-Klett et al., 2011).

It is possible that the enhancement of growth and weight gain by bacteria can be attributed to the fact that they are concentrated in the fruiting bodies, as they work to effectively reduce their enzymes, especially peptidase and lipase enzymes, so they do not decompose the fruiting bodies (Oh and Lim, 2021). The bacteria work to enhance the growth of the edible mushroom *A. bisporus*, especially in the early stages. For growth, which increases the biomass, speed, and size of the fruiting body as a result of the bacteria's secretion of volatile organic compounds (Orban et al., 2023). In addition to their role in reducing the colonization period of hyphae of edible mushrooms in the compost (Suarez et al., 2020), bacteria that promote the growth of edible mushrooms works to inhibit pathogens and decompose their walls, and thus edible mushrooms can benefit from decomposed materials as well (Orban et al., 2023).

The increase in hardness under infection conditions can also be attributed to the fact that the treatments inhibited the pathogenic fungi, which reduced the rate of respiration resulting from the infection. Therefore, the growth rate will decrease and the growth of spores in the gills will be delayed, with the cap not opening, and thus the caps will remain in the form of a cohesive mass (Gortari et al., 2018). It is also possible that treatment with bacteria and biochar can support and enhance the resistance to edible mushroom and increase the hardness of the fruiting bodies by providing them with nutrients such as carbon, potassium, and calcium (Sarfraz et al., 2019).

4. Conclusion

It can be concluded from this study that palm biochar, the bacteria *P. psychrophenolicus*, and the yeast *M. pulcherrima* are among the most effective factors in controlling dry bubble disease caused by *L. fungicola* that infects the mushroom *A. bisporus*. In the presence of the pathogenic fungus, the lowest reduction in the severity of disease infection and the highest productivity of the mushroom yield by bacteria and biochar were recorded in the brown mushroom strain, and the highest hardness was recorded for the same treatment in the white mushroom strain, while the highest protein content was found in the fruit bodies of the brown strain in the treatment of palm biochar and yeast in the presence of the pathogenic fungus.

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