The Role of Spent Oyster Substrate Supplemented with Biological Factors and spraying with Spent substrate extract on some Vegetative Growth Characteristics of Cowpea (*Vigna unguiculata* L.)

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Abstract

The experiment was conducted in pots at the research station of the Department of Soil Science and Water Resources - College of Agriculture / University of Diyala during the period from March to May 2025 to evaluate the effect of spent oyster substrates treated with bacteria and yeast on the growth of cowpea plants. The experiment was performed using a completely randomized design (CRD). The experiment included 14 treatments resulting from the interaction of two factors: The first factor was the incorporation of spent oyster substrates with the soil at a ratio of 25%, comprising seven levels: wheat straw treated with *Azotobacter chroococcum* (WSA), wheat straw treated with yeast(WSY), *Suaeda aegyptiaca* treated with *Azotobacter chroococcum* (SAA), *Suaeda aegyptiaca* treated with yeast(SAY), *Phragmites australis* treated with *Azotobacter chroococcum* (PAA), *Phragmites australis* treated with yeast(PAY), and the control (soil only), The second factor involves two levels of spent oyster substrate extract application (Spraying with spent substrate extract S1 and no spraying S2). The data were statistically analyzed and the means of the treatments were compared according to Duncan's multiple coefficient test at a probability level of 0.05. The results showed that the treatment of *Phragmites australiss* substrate with yeast (PAY)and spraying with the spent substrate extract(S1) achieved the highest plant length and Dry Weight of the Shoots system, reaching 43.00 cm plant^{-1}and 12.43 g plant^{-1} respectively. The wheat straw substrate with *Azotobacter chroococcum*(WSA) and spraying with the extract(S1) recorded the Dry Weight of Root system and number of active nodules, reaching 1.81 g plant^{-1} and 20.66 nodules plant^{-1} respectively. All treatments using spent biological substrates (wheat straw, *Phragmites australis* , and *Suaeda aegyptiaca*). showed a significant advantage in increasing cowpea plant growth compared to the control treatment.

Keywords: Spent oyster substrate , Spent substrate extract, Cowpea.

Introduction

The waste released after mushroom crop harvest called spent mushroom substrate The accumulation of this waste has a negative impact on the environment (air and soil pollution) [12], as it becomes a Phragmites australis ground for insects and pests and a source of unpleasant odors [7]. Using traditional methods to destroy it, such as burning, can contribute to greenhouse gas emissions, causing environmental problems and affecting the well-being of society. Therefore, finding a sustainable solution is necessary, as this waste still retains high levels of organic matter and nutrients and can be useful in agriculture animal as feed, or reuse as a

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substrate for another mushroom [14][4]. It can be transformed into a bio-organic soil enhancer that increases its water retention capacity, improves aeration, reduces the need for chemical fertilizers, increases soil fertility, and thus increases plant productivity. This makes its recycling an essential part of sustainable agriculture strategies [7]. Indicated the possibility of using mushroom residues in the growth and germination of bean seeds. Using these residues as a soil amendment resulted in significant differences in germination rate, leaf length and width, as well as bud and root length[13] . It was also observed that plants treated with the consumed medium contained higher percentages of chlorophyll, protein, and total carbohydrates. [6] stated that the spent substrate resulting from oyster mushroom cultivation acts as a biofertilizer and growth stimulant for lentil plants and contributes to the soil's ability to retain water and fix nitrogen. The combined application of 75% SMC and 25% FYM field fertilizer led to a significant improvement in Plant Length (47.8 cm) and number of leaves (45.3 leaves per plant) compared to 40.23 cm and 28.2 leaves per plant, respectively, without the addition. Therefore, this research approach is consistent with one of the United Nations Sustainable Development Goals, including the twelfth goal (responsible consumption and production), by recycling spent substrates and converting it into valuable resources. The study aimed to use a spent oyster substrate enriched with bacteria and yeast as a biostimulant to improve the properties of cowpea plants.

Material and Methods

Experimental Location:

The experiment was conducted at the research station of the Department of Soil Science and Water Resources, College of Agriculture, University of Diyala, from March to May 2025 to evaluate the effect of depleted substrates resulting from oyster mushroom production treated with bacteria and yeast in cowpea plants**.**

Source of the substrate:

The Spent oyster substrates used in this experiment were producced at the National Center of Organic Farming / Plant Protection Directorate / Ministry of Agriculture - Baghdad The spent oyster substrates used were wheat straw , the succulent plant (Suaeda aegyptiaca) and Phragmites australis grass (Phragmites australis). during the vegetative mushroom(*Pleurotus sapidus*) stageafter These substrates were supplemented with a bio-supplement consisting of bacteria (*Azotobacter chroococcum*) 15 ml was added to each culture bag, The second treatment was baker's yeast (*Saccharomyces cerevisiae*), 50 cm^3 was added to each culture bag and after harvesting of fruiting bodies and used substrates in this experiment.

Experimental Factors:

The experiment was performed using a completely randomized design (CRD). The experiment included 14 treatments resulting from the interaction of two factors: The first factor was the incorporation of spent oyster substrates with the soil at a ratio of 25%, comprising seven levels: wheat straw treated with *Azotobacter chroococcum* (WSA), wheat straw treated with yeast(WSY), *Suaeda aegyptiaca* treated with *Azotobacter chroococcum* (SAA), *Suaeda aegyptiaca* treated with yeast(SAY), *Phragmites australis* treated with *Azotobacter chroococcum* (PAA), *Phragmites australis* treated with yeast(PAY), and the control (soil only), The second factor involves two levels of spent oyster substrate extract application (Spraying with spent substrate

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extract S1 and no spraying S2). Each treatment was repeated three times, resulting in a total of 42 experimental units. Some substrates nutrients were measured as shown in Tables 1 and 2. Nitrogen was measured using a Micro-Kjeldahl apparatus, following the method described [3]. Phosphorus was measured by adding ammonium molybdate and ascorbic acid, and the absorbance was read at a wavelength of 882 nm using a spectrophotometer, according to the method described[10] .Potassium was estimated (%) using a Flame photometry, following the method described [3].

Table 1 Some chemical properties of the bio-substrates.

substrates	N%	P%	K%
WSA	1.33	0.38	1.61
WSY	1.25	0.37	1.45
SAA	1.59	0.52	0.31
SAY	1.46	0.50	2.28
PAA	1.33	0.41	0.85
PAY	1.26	0.40	0.82

Table 2 Some nutritional elements of the substrate extract .

substrate extract	N%	P%	K%
S1	1.33	0.38	1.61

Preparing the substrates and planting cowpeas:

After the oyster mushroom production was completed, the spent substrates were collected and air-dried in the sun , then ground into a homogeneous powder using an electric grinder. Plastic pots capacity of 5 kg were used, the powder of spent oyster substrate was added at a rate of 25% of the soil weight .Five cowpea seeds (*Bayader variety*) were planted in each pot, and the usual agricultural practices of irrigation and weeding were carried out throughout the 60-day growth period. After One month from seeds planting The plant were treated with the extract of the spent substrate which prepared by soaking a quantity of the substrate in water at a ratio of 1:5 for 24 hours at room temperature. Then the extract was filtered, and half of the pots were treated by spraying their plants, while the other half was left without spraying**.**

Studied traits:

Plant Length (cm plant^{-1})

The plant Length in each experimental unit was measured using a measuring tape, and the average was calculated.

Dry Weight of the Shoot system (g plant^{-1})

The plants in each experimental unit were weighed and oven-dried for 24 hours at a constant weight based on the dry weight of the shoot.

Dry Weight of Root system (g plant^{-1})

The plants in each experimental unit were weighed, and the roots were oven-dried for 24 hours at a constant weight based on the dry weight of the root system.

Number of active nodes (plant node $^{-1}$)

The number of nodes formed on the roots of cowpea plants was counted after 60 days of planting by carefully removing the plants from the pots to avoid cutting or losing the roots, then placing them in a sieve and washing them by exposing them to running water to remove dirt. After that, the number of active nodes was counted.

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Statistical analysis

The data was statistically analyzed for all experiments according to Complete Randomized Design (CRD), as they were conducted under controlled conditions to study the effect of various factors on the studied traits, and the significant differences between the means were compared according to the Duncan multinomial test at the probability level of 0.05, which was based on the SAS program for analysis [1].

Results and Discussion

Plant Length (cm plant⁻ ¹)

According to Table 3, the addition of bio-substrates significantly increased Cowpea plant length , all substrates showed a notable increase in plant length when the extract was sprayed. The highest plant height, 43.00 cm plant^{-1}, was observed with the PAY substrate, with no significant difference from WSA, WSY, and SAA. In both extract-sprayed S1 and non-sprayed treatments S2, the control treatment had the lowest plant length, measuring 26.60 and 22.30 cm plant^{-1}, respectively. The increase in plant length may be attributed to the spent substrate containing nutrients Table 1, and its addition to the soil as a fertilizer led to the release of nutrients (K, P, N) readily available to the plant, which positively impacted plant length . [9] found that low concentrations (5%–25%) of the spent substrate led to an increase in plant length, number of leaves, total leaf area, and total chlorophyll content.

Table 3 .Effect of bio-substrates and spraying with spent substrate extract on Plant Length (cm plant^{-1}).

Bio-Substrate Treatments	S1	S2	Averages of bio-substrates
WSA	42.66 a	38.13 b	40.40 a
WSY	39.00 ab	32.33 cd	35.66 bc
SA A	39.33 ab	36.33 bc	37.83 ab
SAY	36.36 bc	28.16 ef	32.26 d
P AA	37.36 b	32.00 de	34.68 cd
PAY	43.00 a	33.03 cd	38.01 ab
control (Soil Only)	26.60 f	22.30 g	24.45 e
Averages of Substrate extract	37.76 a	31.75 b	

Means within a column, row and their interactions followed with the same letters are not significantly different from each other according to Duncan multiple ranges test at significant level of 5%.

The Shoot Dry Weight (g plant⁻ ¹)

Results in table 4 show that the addition of bio-substrates led to a significant increase in cowpea shoot dry weight when sprayed with the substrate extract with the exception of SAY. However, the PAY with extract spraying recorded shoot dry weight of 12.43 g plant^{-1}, which was not significantly different from WSA under extract spraying, which recorded 11.77 g plant^{-1}, compared to the control which recorded 7.49 and 6.90 g plant^{-1}under sprayed and non-sprayed conditions,

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respectively. in addition to an increase in dry vegetative and root mass in cowpea .The increase in Dry Weight of the Shoot system may be attributed to the spent substrate extract may be attributed to its nutrients content .[2, 9] concluded that the spent substrate of oyster mushrooms and plant waste is characterized by its high content of nutrients necessary for plant growth.

Table 4 shows the effect of bio-substrates and spraying with the spent substrate extract on the Shoot Dry Weight (g plant^{-1}).

Bio-Substrate Treatments	S1	S2	Averages of bio-substrates
WSA	11.77 ab	10.21 bc	10.99 a
WSY	10.34 bc	9.05cde	9.70 bc
SAA	10.46 bc	8.18 def	9.32 bc
SAY	8.20 def	8.07 def	8.14 de
PAA	9.79 cd	7.68 ef	8.73 cd
PAY	12.43 a	8.47 def	10.45 ab
control (Soil Only)	7.49 ef	6.90 f	7.19 e
Averages of Substrate extract	10.07 a	8.36 b	

Means within a column, row and their interactions followed with the same letters are not significantly different from each other according to Duncan multiple ranges test at significant level of 5%.

Dry Weight of the Root system (g plant-1)

The results presented in Table 5, the addition of bio-substrates significantly increased the root dry weight system in cowpea when spraying the substrate extract, compared to the control group, with the exception of SAY. However, the addition of biological substrates WSA and SAA, and WSY resulted in a significant increase when the extract was non - sprayed. Spraying the extract led to a significant increase in dry root mass weight in WSA, WSY and PAY substrates, compared to the control group without spraying. However, spraying the extract resulted in the highest root mass weight, reaching 1.81 g plant^{-1}, while the control group, both extract-sprayed S1 and non-sprayed S2, recorded the lowest 0.59, 0.55 g plant^{-1} respectively. The increase in Dry Weight of the Root system can be attributed to the use of spent wheat straw-yeast substrate, as it contains growth hormones that contribute to improving the soil properties that supply the plant with growth hormones such as auxins and indole acetic acid, which are reflected positively on the root system. [5, 8] The possibility of using the spent substrate as a bio-fertilizer and soil improver due to its richness in nutrients and active compounds and its low content of heavy metals.

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Table 5. Effect of bio-substrates and spraying with spent substrate extract on Dry Weight of the Root system (g plant^{-1})

Bio-Substrate Treatments	S1	S2	Averages of bio-substrates
WSA	1.81 a	0.90 de	1.36 a
WSY	1.23 bc	0.85 def	1.04 b
SA A	1.09 bcd	0.87 de	0.98 bc
SAY	0.84 def	0.78 efg	0.81c
PA A	0.97 cde	0.77efg	0.87 bc
PAY	1.31 b	0.80 d-g	1.06 b
control (Soil Only)	0.59 fg	0.55 g	0.57 d
Averages of Substrate extract	1.12 a	0.79 b	

Means within a column, row and their interactions followed with the same letters are not significantly different from each other according to Duncan multiple ranges test at significant level of 5%.

Number of Active root Nodules

As shown in Table 6, the addition of bio-substrates significantly increased the number of active root nodules in cowpea plants under both the extract-sprayed and non-sprayed conditions, except for SAA and SAY, regardless of extract spraying. However, WSA and extract spraying recorded the highest number of active nodules, reaching 20.66 (nodes plant^{-1}), which is not significantly different from P A Y and extract spraying , which recorded 19.66(nodes plant^{-1}) while the control group recorded7.33, 6.00 (nodes plant^{-1}), in sprayed and non-sprayed conditions, respectively. The increased in cowpea the number of active root nodules This may be attributed to the fact that the spent substrate contains decomposed organic matter and nutrients that provide a suitable environment for the growth and reproduction of beneficial microorganisms, including Rhizobia bacteria. This leads to improved root system growth, increased bacterial infection efficiency, and nodule formation. [2] demonstrated that adding 5% of the spent substrate resulted in the highest number and weight of active nodules in cowpea plants

Table 6. shows the effect of bio-substrates and spraying with the spent substrate extract on the number of active root nodules (plant node $^{-1}$).

Bio-Substrate Treatments	S1	S2	Averages of bio-substrates
WSA	20.66 a	19.33 abc	20 .00 a
WSY	17.00 cd	15.66 d	16.33 b
SA A	9.00 efg	6.66 gh	7.83 de
SAY	9.66 ef	7.66 fgh	8.66 d
PAA	10.33 e	19.00 abc	14.66 c
PAY	19.66 ab	18.00 bcd	18.83 a
control (Soil Only)	7.33 fgh	6.00 h	6.66 e
Averages of Spent Substrate extract	13.38 a	13.19 a	

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Means within a column, row and their interactions followed with the same letters are not significantly different from each other according to Duncan multiple ranges test at significant level of 5%.

Conclusion

All substrate s using spent biological substrates (wheat straw, *Phragmites australis* , and *Suaeda aegyptiaca*). showed a significant advantage in increasing cowpea plant growth compared to the control treatment . We recommend the possibility of using the spent substrate as a fertilizer in the growth of cowpea plants and studying it on other plants.

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References

[1] Al-Rawi, K M and Khalaf Allah, Abdul Aziz. 1980. Book: Design and Analysis of Agricultural Experiments, College of Agriculture and Forestry - University of Mosul.

[2] Elsakhawy, Tamer, Muneera D. F. ALKahtani, Ali A. H. Sharshar, Kotb A. Attia, Yaser M. Hafez, and Khaled A. A. Abdelaal.(2020). "Efficacy of Mushroom Metabolites (Pleurotus ostreatus) as A Natural Product for the Suppression of Broomrape Growth (Orobanche crenata Forsk) in Faba Bean Plants" Plants 9, no. 10: 1265. https://doi.org/10.3390/plants9101265.

[3] Haynes, R. J. 1980. A comparison of two modified Kjeldahl digestion techniques for multi- element plant analysis with conventional wet and dry ashing methods. Communications in Soil Science and Plant Analysis, 11(5), 459-467. https://doi.org/10.1080/00103628009367053

[4] Hoa, H. T., Wang, C. L., & Wang, C. H. (2015). The Effects of Different Substrates on the Growth, Yield, and Nutritional Composition of Two Oyster Mushrooms (Pleurotus ostreatus and Pleurotus cystidiosus). Mycobiology, 43(4), 423–434. https://doi.org/10.5941/MYCO.2015.43.4.423

[5] Khalil, S., Panda, P., Ghadamgahi, F., Barreiro, A., Rosberg, A. K., Karlsson, M., & Vetukuri, R. R. (2024). Microbial potential of spent mushroom compost and oyster substrate in horticulture: Diversity, function, and sustainable plant growth solutions. Journal of Environmental Management, 357, 120654.

[6] Kumar, M., Pakpu, T., & Rathore, D. K. . (2023). Effect of spent mushroom compost and farm yard manure on growth and yield parameters of lentil (Lens culinaris L.). International Journal of Advanced Biochemistry Research, SP-7(2124-127 https://doi.org/10.33545/26174693.2023.v7.i2Sb.199.

[7] Luo, J., & Chen, L. (2024). Status and development of spent mushroom substrate recycling: A review. Journal of the Air & Waste Management Association, 74(12), 843–860. https://doi.org/10.1080/10962247.2024.2410447

[8]Martín, C., Zervakis, G. I., Xiong, S., Koutrotsios, G., & Strætkvern, K. O. (2023). Spent substrate from mushroom cultivation: exploitation potential toward various applications and value-added products. Bioengineered, 14(1), 2252138.

[9] Michael, W. K., Tawia, O. G., & Korley, K. N. (2022). Effect of Spent Mushroom Compost of Pleurotus eous Strain P-31 on Growth Performance and Nodulation of Cowpea (Vigna unguiculata Walp.). Tropical life sciences research, 33(3(,129.

[10] Olsen, S. R., & Sommers, L. E. (1982). Phosphorus. In A. L. Page, R. H. Miller, & D. R. Keeney (Eds.), Methods of soil analysis: Part 2. Chemical and microbiological properties (2nd ed., Agronomy Monograph No. 9, pp. 403–430). American Society of Agronomy and Soil Science Society of America.

[11] Owaid, M. N., Abed, I. A., & Al-Saeedi, S. S. S. (2017). Applicable properties of the bio-fertilizer spent mushroom substrate in organic systems as a byproduct from the cultivation of Pleurotus spp. Information Processing in Agriculture, 4(1), 78-82.

[12] Parihar, N. S., Sabharwal, U., & Bisen, J. (2026). The role of spent mushroom substrate in sustainable agriculture environmental management and circular economy development. Discover Sustainability. https://doi.org/10.1007/s43621-026-02596-0

[13] Verma, D., Didwana, V., & Maurya, B. (2020). Spent mushroom substrate: A potential sustainable substrate for agriculture. International Journal of Grid and Distributed Computing, 13(2), 104–109

[14] Ye, D., Hu, Q., Bai, X., Zhang, W., & Guo, H. (2023). Increasing the value of *Phragmites australis* wheat straw in a sustainable integrated agriculture model (SIAM) comprising edible mushroom cultivation and spent mushroom substrate compost. Science of the Total Environment, 869, 161807.

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