

Research Article

Evaluation of black tea waste addition and inoculation with symbiotic fungi on sunflower (*Helianthus annuus* L.) crop traits

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Abstract:

The aim of this study is to understand the importance of mycorrhizal and black tea waste on yield parameters and active compounds for sunflowers instead of chemical fertilizers, which are considered harmful to human health. The experiment was conducted in the Hindiya area / Karbala / Iraq during the 2024 agricultural season at a latitude of 34.56 degrees, a longitude of 44.45 degrees, and an elevation of 24 meters above sea level. The experiment was designed according to the complete randomized block design as a factorial experiment ($2 \times 7 \times 3$). Sunflower seeds were planted in the spring season on 4/3/2024 in rows. After 20 days of emergence, the plants were thinned to one plant per hole, and **black tea waste** was mixed with the soil before planting.

The results indicated a significant effect among the studied treatments, as treatment M3 excelled in the number of seeds in the disk (seed disk-1), weight of 100 seeds (g), biological yield (Mgha-1), oleic acid %, linoleic acid %, and thymol % by injecting the soil with **mycorrhizal**, yielding the highest average of 1783 seed disk-1, 64.06 g, 78.07 Mgha-1, 7.83%, 32.03%, and 8.79%, respectively. While black tea extract treatment T3 excelled in the number of seeds in the disk (seed disk-1), weight of 100 seeds (g), biological yield (Mgha-1), oleic acid %, linoleic acid %, and thymol %, with the highest averages of 1745 seed disk-1, 62.40 seed disk-1, 77.90 Mgha-1, 7.36%, 31.79%, and 7.88%, respectively.

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Introduction

In response to the growing global shift toward sustainable agriculture and the reduction of chemical input dependency, the use of biostimulants and botanical extracts has gained increasing attention for their role in enhancing crop growth and productivity. Sunflower (*Helianthus annuus* L.), a key oilseed crop of significant economic importance, is particularly sensitive to soil fertility and nutrient availability. Recent approaches have highlighted the potential of natural biostimulants as effective tools for promoting the growth and development of sunflowers under various agro-ecological conditions [1]. The application of mycorrhizal fungi has emerged as a prominent strategy within sustainable agriculture due to their symbiotic association with plant roots, which significantly enhances nutrient and water uptake—particularly under conditions of nutrient deficiency or abiotic stress.

1. **Enhancement of Nutrient Uptake** Mycorrhizal fungi are especially effective in improving the absorption of phosphorus, alongside essential micronutrients such as zinc and copper. Their extensive hyphal networks serve as natural extensions of the root system, thereby increasing the surface area available for nutrient and water absorption.
2. **Improved Plant Tolerance to Environmental Stresses** These fungi play a critical role in mitigating the effects of drought, salinity, and poor soil conditions by regulating water uptake and maintaining osmotic balance. Mycorrhizal colonization is known to activate stress-responsive pathways in plants, thereby improving their resilience to adverse environmental conditions.
3. **Stimulation of Plant Growth and Productivity** Mycorrhizae contribute to increased vegetative and root biomass, resulting in enhanced crop yields and improved fruit quality. This effect is attributed to better nutrient availability and hormonal signaling pathways triggered by the symbiosis.
4. **Improvement of Soil Structure and Microbial Activity** The fungal

mycelium aids in binding soil particles, enhancing soil aggregation, aeration, and water-holding capacity. Furthermore, the presence of mycorrhizae promotes a favorable environment for other beneficial soil microorganisms, leading to improved soil health and sustainability [2]. By extending plant roots through the hypha network, mycorrhizae expand the area that can absorb nutrients. Increasing the stress tolerance of plants lessens the effects of environmental stressors like poor soil, salinity, and drought. aids in controlling how much water the soil absorbs. Increasing output and growth: It boosts the crop's root and vegetative growth. It boosts output and enhances fruit quality. Enhancing the structure of soil: By binding soil particles, mycelium improves the soil's ability to retain water and aerate. promotes the soil's beneficial microbial activity [3].

Furthermore, one of the natural substances high in polyphenols and micronutrients that has demonstrated the capacity to promote plant growth, enhance germination, and boost disease resistance is black tea extract [4]. Black tea waste has shown promising potential as a natural biostimulant, contributing to plant growth and soil health through its bioactive

components:

1. Stimulation of Plant Growth

Black tea waste is rich in polyphenols, caffeine, and tannins, which possess antioxidant properties. When applied at low concentrations, these compounds can stimulate seed germination and enhance early plant development, promoting more vigorous growth [5].

2. Enhancement of Soil Microbial Activity

The addition of black tea extract to soil may stimulate beneficial microbial communities, thereby improving nutrient cycling and availability. This activity contributes to a healthier rhizosphere, which supports sustainable crop development.

3. Suppression of Soil-Borne Pathogens

Certain compounds in black tea exhibit antifungal and antibacterial activity, offering a degree of biocontrol against root diseases. This can potentially reduce reliance on synthetic fungicides, aligning with the goals of environmentally friendly agriculture.

Integrative Use with Mycorrhizal Fungi

The combined application of mycorrhizal fungi and black tea waste represents an innovative and sustainable approach to improving the morphological and physiological performance of crops such as sunflower. This dual treatment may lead to:

- Enhanced nutrient and water uptake
- Improved plant vigor and yield
- Strengthened resistance to environmental stressors

·Improved soil fertility and microbial diversity

Such integrative strategies are in line with modern sustainable agricultural practices, offering natural, safe, and effective alternatives to chemical inputs [6].

Material and method

At a latitude of 34.56 degrees, a longitude of 44.45 degrees, and an elevation of 24 meters above sea level, the experiment was carried out in the Hindiya region of Karbala, Iraq, during the 2024 growing season. Getting the experimental soil ready: To prepare the field, till and smooth the soil with a mechanical plow. Create the treatments and carry out the study [7].

experiment design

Concentrations	Treatments
mycorrhizal) 80 mL(	(M1)
mycorrhizal mL.L) 200(	(M2)
mycorrhizal) mL.L 250(	(M3)
mixing of Tea wastes with soil with 30 g/kg.soil	T1)(
mixing of Tea wastes with soil with 50 g/kg.soil	(T2)
mixing of Tea wastes with soil with 100 g/kg).soil	T3)(
without adding any extract and mycorrhizal	Control

The experiment was designed according to the complete randomized block design as a factorial experiment ($2 \times 7 \times 3$) with an experimental unit area of 1 m² and a distance of 35 cm between each unit [8].

Isolation of black tea waste: Black tea was extracted by boiling, placing water in a beaker on a hot plate at a temperature of 100 degrees until boiling, then adding the tea for a quarter of an hour. After that, the tea was filtered by separating the tea waste from the extract, and the tea waste were used in the experiment by mixing the tea waste with the soil before planting [9].

Sunflower seeds were planted in the spring season on 4/3/2024 in rows, with a distance of 75 cm between rows and 25 cm between holes, resulting in four rows in the experimental unit to achieve a plant density of 53,333 plants per hectare. At a depth of 3 cm, 10 seeds were placed in each hole. After 20 days of emergence, the plants were thinned to one plant per hole, and black tea waste were mixed with the soil before planting.

mycorrhizal were injected into the rhizosphere, which is the area near the roots, 30 days after planting.

And after completing the pollination process, the flower heads were covered with complete bags to protect them from birds. Harvesting operations were conducted on 24/7/2024 when the crop reached full ripeness, as indicated by the background of the heads turning yellow and the bracts turning brown.

The studied traits

The characteristics of the yield and its components

1: The number of seeds in the disk (seed disk⁻¹) The number of seeds contained in each disk, both filled and empty, was counted manually. 2: Weight of 100 seed (g).

Seeds from five discs of plants from each experimental unit were mixed, and 100 seeds were randomly counted by hand and weighed on a sensitive scale.

3: Biological yield (Mgha⁻¹)L: The biomass represents the weight of all parts of the plant above the soil surface, including the leaves, seeds,

Determination of active compounds in oils using High-Performance Liquid Chromatography (HPLC) technique:

25 microlitres of the prepared solution were taken and injected into the (HPLC) device for the diagnosis and estimation of the active compounds to be measured (oleic acid, linoleic acid, and thymo) in the leaves. The compounds were separated and their types were determined by comparing them with standard materials on the separation column and under the same conditions and concentrations of the separated materials in the examined sample, The standard model was obtained from Sigma International Trading Company.

Sigma), and the retention time and solution area for the standard model were measured as shown in Table (1)[10,11]

Table 1: Retention time and solution area for some active substances in the leaves.			
Namber standard	standard	Retention time	Area
1	oleic acid	1.54	17568
2	linoleic acid	1.63	16457
3	linoleic acid	2.86	15764

statistically analyzed

The results were statistically analyzed using the Gestate program to distinguish the differences between the treatments according to the complete randomized block design (RCBD) for factorial experiments, and the arithmetic means were compared using the least significant difference (LSD) at the 0.05 level.

Results

Based on the results of Table 2 indicated a significant effect among the studied treatments, as treatment M3 excelled in The number of seeds in the disk (seed disk-1) by injecting the soil with **mycorrhizal**, yielding the highest average of 1783 seed disk-1 , while the lowest average was

observed in the control treatment with 1299 seed disk-1. The results of the same table also indicated a significant effect of the black tea waste mixed with the soil on The number of seeds in the disk (seed disk-1) in the sunflower plant, with treatment T3 outperforming by giving the highest average of 1745 seed disk-1, while the lowest average was 1326 seed disk-1 in the control treatment , While the two-way interaction indicates a significant effect between the two treatments, the treatment (M3 and T3) yielded the highest average interaction with 1956 seed disk-1, while the control treatment recorded the lowest average with 1124 seed disk-1.

Table (2) Effect of mycorrhizal and black tea waste on The number of seeds in the disk (seed disk ⁻¹).					
mycorrhizal ml.L ⁻¹	black tea waste gm\kg				
Control	Control	T1	T2	T3	Mean
	1124	1243	1357	1464	1299
M1	1248	1367	1576	1687	1469
M2	1376	1576	1765	1872	1647
M3	1547	1765	1867	1956	1783
LSD 0.05 for interaction	2.65				
Mean	1326	1488	1641	1745	
	LSD (0.05) blak tea waste gm\k=1.56 LSD 0.05 for mycorrhizal ml.L ⁻¹ =1.44				

Indicate the results of Table 3 to the founds a significant effect among the studied treatments, as treatment M3 excelled in Weight of 100 seed (g) by injecting the soil with **mycorrhizal**, yielding the highest average of 64.06 g , while the lowest average was observed in the control treatment with 56.41g. The results of the same table also indicated a significant effect of the black tea waste mixed with the soil on Weight of 100 seed (g) in the sunflower

plant, with treatment T3 outperforming by giving the highest average of 62.40 seed disk⁻¹, while the lowest average was 57.20 g in the control treatment.

While the two-way interaction indicates a significant effect between the two treatments, the treatment (M3 and T3) yielded the highest average interaction with 67.93 g, while the control treatment recorded the lowest average with 54.76 g.

Table (3) : Effect of mycorrhizal and black tea waste on Weight of 100 seed (g)					
mycorrhizal ml.L ⁻¹	black tea waste gm\kg				
Control	Control	T1	T2	T3	Mean
	54.76	55.84	56.58	58.45	56.41
M1	55.56	56.84	58.83	60.45	57.92
M2	57.84	59.57	61.56	62.75	60.43
M3	60.63	62.84	64.84	67.93	64.06
LSD 0.05 for interaction	2.79				
Mean	57.20	58.77	60.45	62.40	
	LSD 0.05 black tea waste gm\kg=1.47 LSD 0.05 for mycorrhizal ml.L ⁻¹ = 1.85				

Table 4 findings showed that the examined treatments had a significant impact, as treatment M3 excelled on Biological yield (Mgha^{-1}) by injecting the soil with **mycorrhizal**, yielding the highest average of 78.07 Mgha^{-1} , while the lowest average was observed in the control treatment 71.96 Mgha^{-1} . The results of the same table also indicated a significant effect of the black tea waste mixed with the soil on Biological yield (Mgha^{-1}) in the sunflower plant, with

treatment T3 outperforming by giving the highest average of 77.90 Mgha^{-1} , while the lowest average was 72.19 Mgha^{-1} in the control treatment.

While the two-way interaction indicates a significant effect between the two treatments, the treatment (M3 and T3) yielded the highest average interaction with 81.67 Mgha^{-1} , while the control treatment recorded the lowest average with 69.56 Mgha^{-1} .

Table (4): Effect of mycorrhizal and black tea waste on Biological yield (Mgha^{-1}).					
mycorrhizal ml.L^{-1}	black tea waste $\text{gm}\backslash\text{kg}$				
Control	Control	T1	T2	T3	Mean
	69.56	71.86	72.95	73.46	71.96
M1	71.67	73.85	75.97	76.48	74.49
M2	72.75	74.87	77.46	79.98	76.27
M3	74.76	76.86	78.97	81.67	78.07
LSD 0.05 for interaction	2.39				
Mean	72.19	74.36	76.34	77.90	
LSD (0.05) black tea waste $\text{gm}\backslash\text{kg}$	LSD 0.05 black tea waste $\text{gm}\backslash\text{kg}=1.58$ LSD 0.05 for mycorrhizal $\text{ml.L}^{-1} = 1.95$				LSD 0.05 for mycorrhizal ml.L^{-1}

Based on the results of Table 5 indicated a significant effect among the studied treatments, as treatment M3 excelled on Oleic acid% by injecting the soil with **mycorrhizal** yielding the highest average of 7.83% , while the lowest average was observed in the control treatment 3.37% . The results of the same table also indicated a significant effect of the black tea waste mixed with the soil on Oleic acid% in the

sunflower plant, with treatment T3 outperforming by giving the highest average of 7.36% , while the lowest average was 3.79% in the control treatment.

While the two-way interaction indicates a significant effect between the two treatments, the treatment (M3 and T3) yielded the highest average interaction with 9.45% , while the control treatment recorded the lowest average with 1.98% .

Table (5): Effect of mycorrhizal and black tea extract on Oleic acid%.					
mycorrhizal ml.L ⁻¹	black tea waste gm\kg				
Control	Control	T1	T2	T3	Mean
	1.98	2.78	3.87	4.86	3.37
M1	2.64	3.56	5.87	6.56	4.66
M2	4.76	5.45	6.46	8.56	6.31
M3	5.76	7.56	8.56	9.45	7.83
LSD 0.05 for interaction	2.67				
Mean	3.79	4.84	6.19	7.36	
LSD (0.05) black tea waste gm\kg	LSD 0.05 black tea waste gm\kg=1.42 LSD 0.05 for mycorrhizal ml.L ⁻¹ = 1.82				LSD 0.05 for mycorrhizal ml.L ⁻¹

Based the results of Table 6 indicated a significant effect among the studied treatments, as treatment M3 excelled on linoleic acid% by injecting the soil with **mycorrhizal**, yielding the highest average of 32.03% , while the lowest average was observed in the control treatment 25.80% The results of the same table also indicated a significant effect of the black tea waste mixed with the soil on linoleic acid% in the

sunflower plant, with treatment T3 outperforming by giving the highest average of 31.79%, while the lowest average was 25.53% in the control treatment.

While the two-way interaction indicates a significant effect between the two treatments, the treatment (M3 and T3) yielded the highest average interaction with 35.74%, while the control treatment recorded the lowest average with 23.76% .

Table (6): Effect of mycorrhizal and black tea waste on linoleic acid%.					
mycorrhizal ml.L ⁻¹	black tea waste gm\kg				
Control	Control	T1	T2	T3	Mean
	23.76	24.76	26.86	27.83	25.80
M1	24.74	26.87	27.85	30.75	27.55
M2	25.76	28.56	29.76	32.85	29.23
M3	27.86	30.76	33.76	35.74	32.03
LSD 0.05 for interaction	1.86				
Mean	25.53	27.74	29.56	31.79	
LSD (0.05) Black tea waste gm\kg	LSD 0.05 black tea waste gm\kg=1.65 LSD 0.05 for mycorrhizal ml.L ⁻¹ = 1.74				LSD 0.05 for mycorrhizal ml.L ⁻¹

The results of Table 7 indicated a significant effect among the studied treatments, as treatment M3 excelled on thymo% by injecting the soil with **mycorrhizal** bacteria, yielding the highest average of 8.79% , while the lowest average was observed in the control treatment 3.24% The results of the same table also indicated a significant effect of the black tea waste mixed with the soil on thymo % in the sunflower plant, with

treatment T3 outperforming by giving the highest average of 7.88%, while the lowest average was 5.74% in the control treatment While the two-way interaction indicates a significant effect between the two treatments, the treatment (M3 and T3) yielded the highest average interaction with 11.57%, while the control treatment recorded the lowest average with 1.99% .

Table (7) : Effect of mycorrhizal and black tea waste on thymo %.					
mycorrhizal ml.L ⁻¹	black tea waste gm\kg				
Control	Control	T1	T2	T3	Mean
	1.99	2.65	3.76	4.57	3.24
M1	2.67	3.27	5.83	6.83	4.65
M2	3.76	4.76	7.56	8.56	6.16
M3	5.74	8.38	9.45	11.57	8.79
LSD 0.05 for interaction	1.67				
Mean	3.54	4.77	6.65	7.88	
LSD (0.05)	LSD 0.05 black tea waste gm\kg=1.39				LSD 0.05 for
black tea waste gm\kg	LSD 0.05 for mycorrhizal ml.L ⁻¹ = 1.61				mycorrhizal ml.L ⁻¹

Discussion :

According to the findings of recent research, one of the most promising developments in bio-agriculture is the use of plant extracts and mycorrhiza fungi, such as black tea extract, to increase crop productivity and growth while lowering dependency on chemical fertilizers. The impact black tea waste Numerous active ingredients found in black tea, including caffeine, tannins, and polyphenols, have different effects on plant growth. When using the right amounts of black tea waste [12] It could help: Promote vegetative growth by improving nutrient absorption through the action of phenolic compounds,

which could serve as catalysts for the activity of plant enzymes [13].boosting the amount of active substances in plant tissues, particularly flavonoids and phenols, which are antioxidants and improve the quality of seeds and their oils. enhancing quantitative yield characteristics like the weight of a thousand seeds, the number of seeds per head, and the overall seed yield as a result of the extract's natural stimulants' impact on the plant's physiological functions. However, the beneficial effect depends on the extract's concentration; high concentrations may prevent growth because of the harmful effects of some compounds, such as caffeine [14].

The impact of using mycorrhizal fungi for biofertilization

Mycorrhizae are crucial for enhancing yield characteristics in the following ways:

improving the absorption of nutrients, particularly nitrogen and phosphorus, which increases the effectiveness of essential functions like photosynthesis and the production of proteins and oils. increasing the plant's ability to withstand environmental stressors, which promotes steady growth and, in turn, improves yield components.

Because of better mineral nutrition and the activation of the biological pathways that lead to fat formation, sunflower seeds have a higher oil content. Mycorrhiza also increases the concentration of active substances like phenolics and flavonoids in plant tissues, which improves the plant's nutritional and therapeutic value.

According to certain research, mycorrhiza and black tea extract may work in concert to increase each other's efficacy. Mycorrhiza helps to improve the absorption of the extract's active ingredients, while the compounds in tea may increase fungal activity in the soil.

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Conclusion

Increased seed weight and improved oil and active compound content are just a few of the quantitative and qualitative yield traits of the sunflower plant that can be clearly improved by using black tea waste and mycorrhizal fungi. This strategy is a promising first step toward high-productivity, high-quality, sustainable agriculture.

Recommendations

1. Promoting the use of black tea waste as a growth biostimulant because of its beneficial effects on active compounds and yield traits, while taking into account the need to determine the ideal concentration to prevent inhibitory effects.
2. Using mycorrhizal fungi for biofertilization as part of the integrated nutrition management program because it is known to improve nutrient absorption, boost the productivity of sunflower plants, and increase the amount of bioactive compounds.

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