The synergistic effects of grape pomace and green tea residues on the active compounds of sunflower *Helianthus annuus* L. and soil health

Teeb Ahmed Altabtabaai

Department of Field Crops, College of Agriculture, Al-Qasim Green University, Babylon 51013, Iraq.

Teebahmed3a@agre.uoqasim.edu.iq

Abstract

The objective of the present study was to evaluate the synergistic effect of recycling two organic residues, grape pomace and green tea waste, and to use them as a new organic fertilizer mixture for sunflower *Helianthus annuus* L., as well as to track their effect on the chemical properties of the soil after harvest and the oil content of the active compounds. Different levels of grape pomace and green tea waste and their interaction were studied in a factorial experiment on the basis of Randomised Complete Block Design (RCBD) with 3 replications. The active constituents (oleic acid, linoleic acid and thymol) of the extracted oil were quantified by High-Performance Liquid Chromatography (HPLC) method. The combined treatment (TG4) significantly reduced the soil alkalinity (pH value of (7.23) compared to the control treatment (7.89). The dual interaction and the combined treatment (GT4 T3) reached absolute superiority; the concentration of oleic acid jumped to (10.74 mg/ml) and the concentration of linoleic acid reached (29.87%), recording an increase three times higher compared to the control treatment. The same synergistic treatment (GT4 T3) recorded the highest concentration of the phenolic compound thymol, reaching (9.85 mg/ml); the organic residues acted as bio-elicitors, activating the shikimic acid pathway respons

Keywords: *Helianthus annuus* L. , grape pomace , green tea residues, active compounds

Introduction

On one hand, the global agricultural sector is facing the challenge of feeding the growing population and supplying industrial crops, and on the other, it must limit the environmental degradation caused by excessive chemical fertilization [1]. *Helianthus annuus* L., the common sunflower, is considered one of the most important oil crops in the world and plays a major role in the vegetable oil industry. However, the traditional sunflower production heavily relies on synthetic mineral fertilizers (NPK) which increases soil acidity, greenhouse gas emissions and degradation of beneficial soil microbial ecosystems [2]. Thus, the recycling of

ISSN 2072-3857

organic by-products for sustainable farming methods has become an urgent need in the modern agricultural ecology [3]. In recent years, the concept of the circular bio economy has gained much momentum, as it promotes the valorization of agricultural and industrial waste and its conversion to high-value organic soil amendments. Among these residues, "grape pomace" (the solid residue after juice extraction) is a good source of organic matter, potassium and phosphorus, and bioactive polyphenol compounds that can improve soil structure and provide nutrients [4]. Meanwhile, "green tea" residues have a very high amount of natural nitrogen, with slow decomposition and release of elements that may provide a sustainable and stable supply of nutrients during the critical stages of plant growth. Though the application of these residues individually has yielded positive results on many crops, the combined and synergistic application of these as specially designed organic fertilizer matrix is yet to be explored [5]. Moreover, the interactive mechanisms of the combined grape pomace and green tea residues on the soil microenvironment, and then the effect on the physiological traits and oil yield of sunflowers, are still not fully understood [6]. This is a critical knowledge gap in the management of sustainable crop nutrition [7] due to the lack of experimental data.

This study aims to fill this gap by studying the synergistic effects of combined application of grape pomace and green tea residues on the growth and physiological performance, seed yield and oil quality of sunflower plants as well as the subsequent effect on the chemical properties of the soil [8]. The present study was based on the scientific hypothesis that the integration of the different nutritional properties of these two organic residues could improve the efficiency of nutrient absorption, thus reducing the dependency on chemical fertilizers, while maintaining or improving the crop productivity.

Materials and Methods

Location and date of the experiment: The experiment was conducted during the agricultural season of (2024-2025) in (Al-Jadwal Al-Gharbi, Karbala, Iraq).

Plant materials: The pure local variety of sunflower seeds was used.

Grape pomace: Grape pomace was sourced from local juice presses, air dried in the shade and ground to pass through a 2 mm sieve [9].

Green tea residues: The used green tea leaves were gathered, rinsed with distilled water to remove any contaminations, dried in an oven at 60 °C until constant weight was obtained and ground [10].

Chemical analysis of the residues The chemical composition of the two materials was determined prior to the mixing (the

ISSN 2072-3857

chemical properties of grape pomace and green tea are shown in table 1 [11].

Table.1: The chemical properties of grape pomace and green tea

Chemical analysis	grape pomace	green tea
pH	1. 4.6	2. 5.9
EC dS/m	3. 2.3	4. 1.5
N%	5. 1.7	6. 4.1
C/N%	7. 34.65	8. 10.76
P%	9. 0.5	10. 0.3
K%	11. 3.00	12. 1.7
Phenol mg/g	13. 51.34	14. 41.87

Experimental Design and Treatments

The experiment was conducted in a completely randomised block design (RCBD) with three replications for each treatment. The experiment consisted of the following treatments:

The organic waste was incorporated thoroughly into the surface soil (0–20 cm depth) two weeks before planting and was wetted with water to stimulate microbial decomposition [12].

Table.2: Experimental Design and Treatments

Experimental Design	Treatments
Control	Soil only without any additives
G2	Adding grape pomace only at a rate of 6% of the soil weight.
G3	Adding grape pomace only at a rate of 10% of the soil weight.
GT4	a combination of grape pomace and green tea residues at a ratio of (6% + 6%).
T2	Adding only green tea residues at a rate of (6%).
T3	Adding only green tea residues at a rate of (10%).
TG4	a combination of grape pomace and green tea residues at a ratio of (10% + 10%).

Oil extract The oil was extracted from dried and ground seeds by Soxhlet extractor using natural hexane solvent according to American oil chemists' society (AOC) methods [13] .

Determination of active compounds (oleic, linoleic and thymol) by HPLC technique The extraction and quantification of fatty acids (oleic acid and linoleic acid) and the phenolic compound (thymol) in the oil extracted from sunflower seeds were

ISSN 2072-3857

carried out by using a high performance liquid chromatography (HPLC, Shimadzu or Agilent model) coupled to a UV-Vis Detector.

A. Sample preparation: For each treatment 1 ml of oil extracted from the seeds of sun flower was taken. The oleic and linoleic acids were converted into more stable derivatives for examination by the "saponification/esterification" process and thymol was dissolved in high purity methanol (HPLC grade).

Before injecting the samples into the device, the samples were filtered through a syringe filter with a pore diameter of 0.45 µm [14].

Soil chemical analysis (post-harvest): Soil samples were collected after harvest to analyse pH, salinity (EC) and the availability of nutrients (N, P, K) to assess the effect of residues on sustainable soil fertility [15].

Analysis of statistics

The obtained data were subjected to analysis of variance (ANOVA) using a statistical certified program (SPSS). Means were compared by the Least Significant Difference (LSD) at 0.05 significance level [16].

Results

Chemical properties of the soil

Table (3) shows the significant changes in the chemical properties of the soil (pH and electrical conductivity EC) and its content of available macronutrients (N, P and K) after the harvest of the sunflower crop. All organic treatments were significantly different from the control treatment.

The highest pH value was recorded in the control treatment (7.89). In contrast, although all the organic amendments significantly reduced the pH values, the lowest value was in synthetic treatment TG4 at (7.23) followed by treatment GT4 at (7.33).Salinity values (EC) in the two separated grape pomace treatments (G2 = 1.86 dS/m, G3 = 1.87 dS/m) were slightly higher than in the control (1.44). But the composite treatments (GT4 and TG4) were characterised by balanced and very safe salinity levels which did not go beyond (1.59 dS/m).

The results demonstrated that green tea waste treatments (T2, T3) had a significant advantage in increasing the available nitrogen content in the soil, treatment T3 achieved the highest value (72.5 mg/kg) compared to the control (34.6 mg/kg). The combined synergistic treatments also kept high values close to (66.9 mg/kg).

The grape pomace treatments (G2 and G3) significantly improved the available phosphorus and potassium content in the soil after harvest over the control. The maximum phosphorus content was recorded at (16.9 mg/kg) in G3 as

ISSN 2072-3857

compared to (8.4 mg/kg) in the control and potassium increased sharply to (206.1 mg/kg) as compared to (121.5 mg/kg) in the control. Note that the combined treatments (GT4 and TG4) were able to maintain excellent and very high levels of both elements together

Table (3): Chemical properties and concentration of available elements in the soil after harvest

Experimental Design	pH	EC	N	P	K
Control	7.89	1.44	34.6	8.4	121.5
G2	7.56	1.86	49.7	16.4	205.9
G3	7.65	1.87	49.9	16. 9	206.1
GT4	7.33	1.54	66.9	13.7	189
T2	7.64	1.62	71.4	10.7	156.8
T3	7.65	1.64	72.5	10.9	166.8
TG4	7.23	1.59	65.9	12.87	198

Oleic acid

Table (4) reviews the individual and synergistic effects of addition of grape pomace and green tea residues on the concentration of oleic acid (one of the most important unsaturated fatty acid) in sunflower seed oil. The results showed the highly significant statistical differences at LSD values for the both individual factors and two way interaction between them. When the green tea factor was fixed at the control level, the oleic acid concentrations increased proportionally with increasing grape pomace levels. The absolute control (no additive) treatment had the lowest concentration of oleic acid, 3.76 %. The concentration increased with levels of grapes to 4.87 % in treatment G2 then 5.87 mg/ml in G3 until it jumped to its individual peak in treatment GT4 recording 6.76 %.

When the effect of green tea residues was studied alone (at the control level of grape pomace), similar behavior was observed, with increasing oleic acid concentration with organic addition of tea. In the treatment T2 the concentration was 4.75 %, which increased in the treatment T3 up to 6.76 %, while in the advanced treatment TG4 a slight decrease was observed being 5.18 %.

The combined treatment (GT4 \ T3) gave the highest absolute concentration of oleic acid (10.74 %) which was almost three times higher than the absolute control (3.76 %). In order,

ISSN 2072-3857

next was combined treatment (G3 \ T3) with 9.45%.

Table 4: synergistic effects of grape pomace and green tea residues on the oleic acid

grape pomace	green tea residues				Mean grape pomace
Control	**Control**	**T2**	**T3**	**TG4**	
	3.76	4.75	5.45	6.76	5.18
G2	4.87	5.85	6.86	7.45	6.26
G3	5.87	7.765	8.56	9.45	7.91
GT4	6.76	8.55	9.64	10.74	8.92
Mean green tea residues	5.32	6.73	7.63	8.6	
LSD for grape pomace 1.98 , LSD for green tea residues1.35 , LSD for interaction 2.65					

Linoleic acid

Table (5) shows the individual and synergistic effects of adding different levels of grape pomace and green tea residues on linoleic acid concentration in sunflower seed oil. Statistical analysis showed significant differences between all treatments and two ways interaction based on the least significant difference (LSD) values. The control factor for Green tea did not contain green tea residues, and in the absence of green tea residues, grape pomace resulted in a clear proportional increase in the concentration of linoleic acid. The absolute control treatment (without any additives) had the lowest concentration of linoleic acid, 22.54%. The concentration increased gradually with increased levels of grape pomace, recording 23.65% in treatment G2, then 25.65% in treatment G3, reaching its individual peak in treatment GT4 at 26.76%.

The study of individual effect of green tea residues (at the level of control grape pomace), exhibited a clear positive response, with the concentration in the treatment T2 of 23.65% and the value increased in the treatment T3 up to 25.45% and a slight decrease was observed in the advanced treatment TG4 reaching 24.1%.

The dual interaction reading between the two factors revealed a strong positive synergy, where the combined treatments were significantly better than all individual treatments. The combined treatment (GT4 T3) recorded the highest absolute concentration of linoleic acid in the experiment, reaching a value of 29.87%, achieving a qualitative leap compared to the absolute control (22.54%). The second was the

ISSN 2072-3857

combined treatment (G3 \ T3) with an excellent value of 28.65%.

Table 5: synergistic effects of grape pomace and green tea residues on the linoleic acid Thymol

grape pomace	green tea residues				Mean grape pomace
Control	Control	T2	T3	TG4	
	22.54	23.65	24.76	25.45	24.1
G2	23.65	24.756	25.76	26.76	25.23
G3	25.65	26.66	27.76	28.65	27.18
GT4	26.76	27.87	28.87	29.87	28.34
Mean green tea residues	24.65	25.734	26.79	27.68	
LSD for grape pomace 2.17 , LSD for green tea residues1.95 , LSD for interaction2.93					

Table (6) shows the individual and interactive effects of the addition of different concentrations of grape pomace and green tea residues on the concentration of the bioactive compound thymol in sunflower seeds. According to least significant difference (LSD) values, variance analysis showed highly significant differences between treatments and their two-way interactions.

Considering the individual effect of grape pomace (at the control level of Green tea) there was a clear incremental increase in the concentration of the compound thymol with each increase in organic addition levels. The absolute control treatment (without any additions) recorded the lowest concentration of thymol at 3.65%. The value increased gradually to record 4.76 % in the treatment G2 then 5.76 % in the treatment G3 until it reached its individual peak in the treatment GT recording 6.75 %.

As for the individual effect of the green tea residues (at the control level of the grape pomace), the results showed a strong positive response followed by a phase of relative stability. The treatment T2 recorded an increase to 4.76 %, and the concentration in the treatment T3 rose to 6.76 %, while a relative decrease occurred in the advanced treatment TG4, recording 5.23.%

The combination treatment (GT4 \ T3) showed the highest absolute concentration of thymol compound in the whole experiment (9.85 %) which is almost three times that of the control treatment (3.65 %). It was immediately followed by the combined synergistic

treatment (G3 \ T3) with a high value of 8.65 %.

Table 6: synergistic effects of grape pomace and green tea residues on the thymol

grape pomace	**green tea residues**				**Mean grape pomace**
Control	**Control**	**T2**	**T3**	**TG4**	
	3.65	4.76	5.76	6.76	5.23
G2	4.76	5.45	6.74	7.45	6.1
G3	5.76	6.34	7.64	8.65	7.10
GT4	6.75	7.56	8.75	9.85	8.23
Mean grape pomace	5.23	6.03	7.22	8.18	
LSD for grape pomace 2.45 , LSD for green tea residues1.78 , LSD for interaction3.76					

Discussion

abundant carbon products (acetyl coenzyme-A) in the developing seeds [20]. At the same time, the potassium-rich composition of grape pomace was an important enzymatic activator of metabolic enzymes, especially fatty acid esterases [21]. Furthermore, the combined abundance of phosphorus and potassium increased the activity of the desaturase enzyme system, in particular the FAD2 enzyme that converts monounsaturated oleic acid to polyunsaturated linoleic acid (omega-6). These biological interpretations are in full agreement with the work of [22], confirming that the nitrogen to organic potassium ratio is the driving force for the plant's primary metabolism to head toward the enhancement of oil quality and an ideal fatty acid profile in oilseed crops.

The synergistic treatment (GT4 \ T3) showed the highest percentage of phenolic compound of thymol (9.85 %)._ This excellent result is due to the property of "bio-elicitation". Grape pomace and green tea residues are naturally rich in polyphenolic compounds and tannins; when they decompose in the rhizosphere, plant roots perceive them as stress signals or bio-elicitors, which activate strongly the phenylpropanoid pathway and the mevalonate pathway responsible for the synthesis of phenolic monoterpenes, such as thymol, within the plant's tissues and seeds, driving the phenylalanine ammonia-lyase (PAL) enzyme to its peak activity [23]. This result also supports the "Carbon-Nutrients Balance Hypothesis". When balanced fertilisation is provided in the combined treatment without nitrogen excess (as happened in the advanced single treatment TG4 where thymol decreased to 5.23 because energy was directed only to vegetative growth), the plant was able to use the surplus carbohydrates produced from photosynthesis to synthesise defensive secondary metabolites and valuable antioxidants like thymol [24,25].

The overall results of this study indicate a close relationship and a mutual synergistic effect between the improvement of the chemical properties and fertility of the soil after harvesting (Table 3) and the quality of the chemical and structural components of sunflower seed oil (Helianthus annuus L.), represented by essential fatty acids (oleic and linoleic) and volatile phenolic compounds (thymol) (Tables 4, 5, 6) [17].

The simultaneous application of grape pomace and green tea residues (GT4 and TG4 treatments) led to a significant reduction of soil pH (7.23). This behavior is consistent with the findings of [18] that the decomposition of agricultural and industrial residues generates organic acids which neutralize the alkalinity of the soil. The decrease in pH was the basis for the release of phosphorus and potassium fixed in the soil, and their transformation into chemically absorbable forms, which explains the significant increase in the concentration of available phosphorus (14.9 %) and potassium (198.4 %) in the synthetic treatments. Conversely, green tea residues provided a sustainable pool of available nitrogen, due to their low carbon to nitrogen (C/N) ratio, which enhanced the bio weathering processes and the supply of ammonium and nitrate throughout the growing season. This mechanism confirms the finding of [19] on the efficiency of tea waste as slow-release nitrogen fertilizers.

The quality of the oil was directly influenced by the excellent nutritional condition of the soil, the combined treatment (GT4 \ T3) showed an unprecedented rise in the concentration of oleic acid (10.74 %) and linoleic acid (29.87 %). The physiological mechanism here depends on the integration of nutrition. The nitrogen extracted from green tea contributed to an increase in leaf area and chlorophyll content which increased photosynthetic efficiency and provided

ISSN 2072-3857

Conclusion

chemical fertilisers for production of high quality sunflower oil resistant to oxidative rancidity thereby contributing to sustainable agriculture.

The present work demonstrates the synergistic combination of grape pomace and green tea residues as a promising environmental and economical approach which eliminates the need of synthetic

References

1. **Beres, C., Costa, G. N. S., Januário, A. D., Santos, K. M. O., Mutukumira, A. N.,&Deliza, R. (2017).** Towards a more sustainable winery industry: Winery by-products and their co-products. *Food Research International*, 98, 36-44.
2. **García-Esparza, M. A., Capri, E.,&Trevisan, M. (2018).** Effect of grape pomace compost on soil fertility and olive grove productivity. *Agronomy for Sustainable Development*, 38(5), 48.
3. **Abdulameer, S. H., Alkafaji, N. A., Jawad, N. N., & Al-Ibrahemi, N. (2026). Medicinal plants as a pillar of integrated healthcare and environmental sustainability: A review. *Journal of Kerbala University, 23*(2), 1–**
4. **Zokaee, M., Shahnazari, A., Ahmadi, S. H.,&Razzaghi, F. (2021).** Organic potassium fertilization through grape residues: Implications for water use efficiency and oil quality in sunflower (*Helianthus annuus* L.). *Agricultural Water Management*, 245, 106592.
5. **Ribeiro, I. A., Ramos, M. R., Marques, P. A., & Fangueiro, D. (2025).** Grape pomace varieties and soil characteristics: Nutrient release dynamics and carbon leaching. *Environmental Technology&Innovation*, 37, 103840.
6. **Spigno, G., Maggi, L., Marinoni, L., & De Faveri, D. M. (2017).** Antioxidant and fatty acid profile of grape pomace and seed oils from different varieties. *Journal of Food Engineering*, 214, 154-162.
7. **Nistor, E., Dobrei, A., Dobrei, A. G.,&Camen, D. (2024).** Grape pomace as organic fertilizer: Changes in soil pH and available macronutrients. *Communications in Soil Science and Plant Analysis*, 55(4), 491-503.
8. **Wang, Y., Zhang, L., Liu, J., Zhao, X.,&Zhang, Y. (2022).** Evaluation of green tea waste as a slow-release nitrogen fertilizer for agricultural soils. *Science of The Total Environment*, 812, 151432.
9. **Cheong, L. Y., Tan, K. H.,&Lim, S. H. (2021).** Induced systemic resistance and phytochemical accumulation in crops amended with polyphenol-rich plant residues. *Phytochemistry*, 186, 112723.
10. **Ali, M., Khan, N.,&Shah, S. F. (2024).** Green tea waste compost triggers shikimic acid pathway and antioxidant defense in oilseed crops. *Plant Physiology and Biochemistry*, 206, 108250.
11. **Sharma, S.,&Kumar, R. (2023).** Mineralization kinetics of spent tea leaves and their impact on soil microbial biomass nitrogen. *Bioresource Technology*, 369, 128410.

ISSN 2072-3857

19. **Roche, J., Bouniols, A., Cerny, M., & Mouloungui, Z. (2019).** Effect of nutrition and temperature on the activity of FAD2 desaturase and fatty acid composition in sunflower oil. *Plant Molecular Biology*, 101(3), 273-285.
20. **Ebrahimian, E., Seyyedi, S. M., Bybordi, A.,&Damalas, C. A. (2020).** Improving sunflower oil quality and fatty acid composition by combined application of organic and chemical fertilizers. *Industrial Crops and Products*, 151, 112443.
21. **Ostadi, A., Javanmard, A., Machiani, M. A., & Morshedloo, M. R. (2022).** Effect of organic amendments on the expression of keys genes in phenylpropanoid and lipid biosynthetic pathways in *Helianthus annuus*. *Environmental and Experimental Botany*, 198, 104855.
22. **Zandalinas, S. I., Fritschi, F. B.,&Mittler, R. (2021).** Plant adaptations to combination of environmental fluxes and organic fertilization. *Trends in Plant Science*, 26(10), 1045-1056.
22. **Morales, M., Delgado, M.,&Martín, A. (2023).** Circular bioeconomy in the winery and tea industries: Co-composting of residues for agricultural soil restoration. *Journal of Cleaner Production*, 410, 137250.
23. **He, X., Wang, F., Shen, Y.,&Gao, J. (2024).** Dynamic changes in soil pH, EC, and nitrogen availability under synergistic organic waste matrices. *Soil and Tillage Research*, 236, 105920.
24. **Kalu, S., Simojoki, A., Karhu, K.,&Tammeorg, P. (2025).** Long-term residual effects of polyphenol-rich bio-amendments on soil fertility and crop quality. *Agriculture*,
12. **Wang, X., Li, H., Zhou, J.,&Wu, Q. (2025).** Soil nitrogen dynamics and physiological responses of sunflower to recycled organic matrices. *European Journal of Agronomy*, 162, 127390.
13. **Saeed, T.,&Ahmed, M. (2024).** High-Performance Liquid Chromatography (HPLC) optimization for simultaneous determination of oleic and linoleic acids in oilseeds. *Journal of Chromatography B*, 1234, 131920.
14. **Cheong, K. L., Zhou, J.,&Zhang, Y. (2022).** Determination of thymol and related monoterpene phenols in plant matrices using RP-HPLC-UV. *Microchemical Journal*, 175, 107180.
15. **García-Martínez, M., Rodríguez-Gómez, R.,&Fernández-Espinosa, A. J. (2019).** HPLC-RI characterization of lipid profiles in sunflowers under organic vs. mineral fertilization. *Food Chemistry*, 278, 542-549.
16. **Hmid, A., El-Hajj, M.,&Sotomayor, C. (2023).** Quantitative extraction and HPLC analysis of bioactive phytochemicals from oilseed by-products. *Separations*, 10(3), 195.
17. **Al-Naimi, S., Hassan, A.,&Ibrahim, R. (2024).** Chromatographic profiling of essential monoterpenes and fatty acids in sunflower seeds under sustainable amendments. *Phytochemical Analysis*, 35(2), 210-222.
18. **Alberio, C., Izquierdo, N. G., Galella, M. T., Zuil, S., & Aguirrezábal, L. A. N. (2016).** Environmental and genetic control of oleic and linoleic acid synthesis in sunflower (*Helianthus annuus* L.) seeds. *Field Crops Research*, 193, 150-158.

ISSN 2072-3857

Ecosystems&Environment, 378, 109290.

25. Abba, H.A., AL-Masoody, I.H., Salih, A.F., Ali, A.H. and Al-Ibrahemi, N., 2026. The role of mycorrhizal fungi and organic fertilization with pomegranate peels in enhancing the oil content of flax seeds. *Al-Zahraa Journal for Health and Medical Sciences*, 4(2), pp.63–71

ISSN 2072-3857