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Research article

New olive-pomace fertilizer tested with a 2-tiers approach: Biomarkers on *Eisenia fetida,* physiochemical effects on *Solanum lycopersicum* and *Olea europaea*

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A B S T R A C T

Every year, the olive oil industry generates a substantial amount of pomace, a semi-solid residue made up of skin, pulp, pit, and kernel fragments. Rather than being disposed of, the pomace can be dried and transported to an extraction facility where pomace oil can be extracted. Utilizing its high thermal capacity, the extracted pomace can be used as a supplementary fuel in the drying process, resulting in the production of ashes. In this study, the effect of pomace waste applied to the soil was investigated by testing two mixtures with different proportions of de-oiled pomace flour and kernel ash (50:50 and 70:30, respectively) in powder and pellet form. We used a dual approach, evaluating the effects of the mixtures on both soil communities and plant physiology and productivity, to assess the actual usability of the fertilizer in agriculture. The biomarker approach was valuable in assessing the sublethal effects of the two mixtures in powder form in soil. After 30 days of exposure, the bioindicator organism *Eisena fetida* showed lipid peroxidation, glutathione S-transferase and lactate dehydrogenase levels similar to the control, while lysozyme activity was reduced in all treatments. The powder mixture was lethal to the tomato plants, while there was no evidence of any damage to the olive trees. During 60 days of monitoring, both mixtures in pellet form showed a slight increase in physiological parameters, suggesting a benefit to the photosynthetic system. The improved carbon assimilation in tomato plants treated with the mixtures results in increased plant productivity, both in terms of number and weight of fruits, while maintaining the antioxidant content. This study paves the way for the use of the pomace mixture as a soil improver, thus increasing the value of this waste product.

1. Introduction

The Mediterranean region is the leading producer of olive oil in the world, accounting for more than 95% of total olive oil production in 2019 (Leone et al., 2021). The economic and nutritional value of olive oil is indisputable; however, the olive industry is constantly challenged by environmental pollution caused by olive mill waste, such as olive mill wastewater and olive pomace. Olive pomace is a semi-solid residual product comprising skin, pulp, stone, and olive kernel, which contains phenols, lipids, and organic acids, all of which are generated and discarded during two- and three-phase extraction systems (Diacono and Montemurro, 2019). Several factors contribute to the difficulties in disposing of olive pomace: the large quantity produced (0.5–0.6 tons for each ton of olives processed) and accumulated in the short period of activity of olive mills, such as the phytotoxic and antimicrobial effects of both phenolic compounds and lipid fractions (Diacono et al., 2012) as well as the acidic pH (Ameziane et al., 2019). Rather than being discarded, pomace can be subjected to extraction processes to obtain crude pomace oil, mainly using hexane as an organic solvent (Sánchez Moral and Ruiz Méndez, 2006). Prior to extraction, the moisture and volatile content of the solid olive mill waste should be reduced during the drying phase due to its high thermal capacity (Sánchez Moral and Ruiz Méndez, 2006), the extracted pomace meets the high energy demand of the drying process (Alonso-fariñas et al., 2020). The use of extracted pomace as a fuel results in the production of ash as a waste product. Ash is typically landfilled near the plants where it is produced, but ash disposal is expensive and subject to strict regulations (Nogales et al., 2011). Although olive mill waste has undeniable economic value, it also raises many environmental concerns. As a result, many studies have been published on the effects of olive mill waste on plants and soils.

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Dry pomace and ashes, properly mixed, could be a good soil improver, but any environmental problem should be excluded. There are few studies on the toxicological effects of olive mill waste and no studies on the toxicological effects of olive mill waste dry pomace and ash. Trigui et al. (2022) studied the effects of olive mill waste at the molecular and organismal levels in the epigeic earthworm *Dendrobaena veneta*. The authors found that oxidative stress caused changes in the levels of the biomarkers acetylcholinesterase (AChE), catalase (CAT), glutathione S-transferase (GST), lipid peroxidation (LPO), and malondialdehyde (MDA) after 7 days of exposure, while enzyme activity recovered in most cases after 28 days. Kovacevic et al. (2022) investigated the effects of olive mill wastewater (OMWW) and olive mill waste contaminated soil (OMW CS) on springtail *F. candida* survival, reproduction, neurotoxicity (AChE), oxidative stress (superoxide dismutase (SOD), GST, and MDA induction), and available energy (lipid and carbohydrate content). The organism was exposed to different ratios of OMWW and OMW CS and the results showed that OMW CS was more toxic in terms of survival and reproduction. Sublethal effects were also observed, including neurotoxicity, oxidative stress and changes in available energy. Under laboratory conditions, Mekersi et al. (2021) investigated the effect of environmentally realistic concentrations of olive mill wastewater and olive mill pomace (12.5%, 25%, 50%, 75%, and 100% w/w) on the growth, reproduction, and survival of the earthworms *Aporrectodea trapezoides* and *Eisenia fetida*. The results showed that *Eisenia fetida* grew faster when exposed to 12.5% OMP, with no effect on reproduction or survival. Mkhinini et al. (2019) exposed *Eisenia andrei* specimens for 7 and 14 days to five agricultural soils irrigated with treated wastewater for 1 year, 8 years, and 20 years. Catalase, glutathione transferase, malondialdehyde accumulation, acetylcholinesterase and the micronucleus test were evaluated. Catalase, GST activity, and LPO increased significantly in all irrigated soils.

Olive mill pomace has also been tested for toxicity to plant species. Leone et al. (2021) studied the effects of composted olive pomace (>50%) combined with sewage sludge, artichoke residues and wheat straw on tomato yield. Although the improved yield was found only when organic fertilizer was combined with mineral fertilizer, no negative effects were reported. Parrotta et al. (2016) studied the effects of different concentrations of olive mill waste on photosynthetic pigments and levels of the enzyme RuBisCO in tobacco leaves, obtaining comparable results in treated and control samples. They also highlighted that treated plants did not show any morphological or structural changes. A field experiment (Proietti et al., 2015) also provided data on solid olive mill waste (SOMW) and composted-SOMW: treatments were evaluated on twenty-year-old trees, and no differences in soil bacterial community, leaf net photosynthesis, or canopy volume were reported; the only exception was the phenol content in the oil, which was higher in the oil produced by treated olives. As mentioned above, the majority of studies have focused on the environmental effects of olive mill waste. The effects were mainly studied using specific model plants and physiological or biochemical traits (Bargougui et al., 2019; Tajini et al., 2020). To improve the sustainability of olive processing and oil extraction, the recovery and reuse of olive waste should be carefully considered as a next step. Indeed, the literature has mainly focused on the use of pomace as a fertilizer in horticulture (Lacolla et al., 2019, 2021), but the potential and properties of olive waste combustion ashes have not yet been investigated.

We hypothesized that de-oiled pomace flour and pomace ash could be used as soil amendments. Due to the lack of clear toxicity effects in the literature, it was necessary to evaluate toxicity on both soil bioindicator organisms and plants. Through a double-approach study, we aimed to assess the actual usability of fertilizer in agriculture by evaluating the effects of the mixtures in pellet formulations on soil communities, plant physiology and productivity. To assess the toxicological effects on soil communities, we used the bioindicator organism *Eisenia fetida*, which was exposed to different pomace flour and dry ash mixtures in artificial soil. A set of biomarkers was used to assess the sublethal effect: LPO, GST, lactate dehydrogenase (LDH), and lysozyme (LYS). The effect on the plants was assessed by applying mixtures of pomace flour and dry ash to tomato and olive plants. We chose small tomato varieties such as San Marzano nano, while the olive research was carried out on 18-month-old plants (cv. Frantoio). Parameters related to photosystem efficiency and leaf gas exchange were studied for both tomato and olive plants. Fruit yield and quality were also studied in tomato plants.

2. Materials and methods

Compost was used in two different formulations with different proportions of pomace flour and pomace ash: 50% pomace flour and 50% pomace ash (Mix 1) and 70% pomace flour and 30% pomace ash (Mix 2). The proportions in the mixtures were selected on the basis of the nutrient content of the flours and ashes. This guarantees the minimum amount of nutrients required in a soil and lowers the potential toxicity of the metals contained in the ashes. The powder of the two mixtures was used to test their potential toxicity in an easily dispersible form. Toxicity was tested on earthworms and on olive and tomato plants. All mixtures were then pelleted by mechanical compression and tested on olive and tomato plants. The experimental design is summarized in Fig. 1.

The presence of heavy metal residues (UNI EN 13657:2004 method + APAT CNR IRSA 3020 Man 29 2003), organochlorine pesticides (OC) and polycyclic aromatic hydrocarbons (PAH) (EPA method 3550C 2007 + EPA 8270 E 2018) were evaluated.

2.1. Eisenia fetida sublethal effects

Eisenia fetida was chosen because it is an epigean earthworm species adapted to decaying organic matter, an excellent soil bioindicator widely used in ecotoxicological studies and already used by the authors to evaluate the toxicological effects of olive pomace (Campani et al., 2017). Each test group consisted of 25 adult earthworms (*E. fetida*) (0.18 g ± 0.06), with well-developed clitellum, kept in glass test containers, covered with a perforated lid, with 1000 g of artificial soil prepared in the laboratory (with 50% w/w organic potting soil and 50% w/w air-dried quartz sand). After one week of acclimatization, they were exposed for 30 days at 20 °C in the dark at the different concentrations of pomace flour and pomace ash as reported in Table 1. The soils were kept moist by wetting them once a week. Moisture, pH, and conductivity values were measured at the beginning and at the end of the experiment.

2.2. Biomarkers analysis

Animals were euthanized by immersion in cold nitrogen and then homogenized with a Potter homogenizer in 0.1 M K-phosphate buffer (100 mg of animal/1 mL buffer). An aliquot of homogenized tissues was used to determine LPO levels. The remaining homogenate was centrifuged at 13,200 g for 30 min at 4 °C and the post mitochondrial fraction (PMS) supernatant was removed and used to determine GST activity, LDH activity, and LYS. LPO was estimated in the whole organism using the procedures of Ohkawa et al. (1979) and Bird and Draper (1984), modified by Campani et al. (2017). Absorbance was measured at 535 nm with an AGILENT Cary UV 60 spectrophotometer, and LPO was expressed as nmol of thiobarbituric acid reactive substances (TBARS) formed per mg protein ($\varepsilon = 1.56 \times 105\ M^{-1}\ cm^{-1}$). GST activity was measured in PMS fraction according to Habig et al. (1974). Briefly, the assay mixture contained K phosphate buffer 0.2 M (pH 7.9) 0.2 mM 2, 4-Dinitrochlorobenzene (DNCB), 0.02 mM reduced glutathione (GSH), and PMS. Enzyme activity was quantified by measuring the conjugation of GSH with DNCB at 342 nm (25 °C) and expressed as nmol DNCB x min^{-1} x mg $protein^{-1}$ ($\varepsilon = 9.6 \times 10^{-3}$ M cm^{-1}). LDH activity was evaluated in the PMS fraction of *E. fetida* according to the technique described by Menezes et al. (2006) and adapted by Caliani et al. (2023). The 96-well microplate was loaded with 3 replicates of 40 μL of

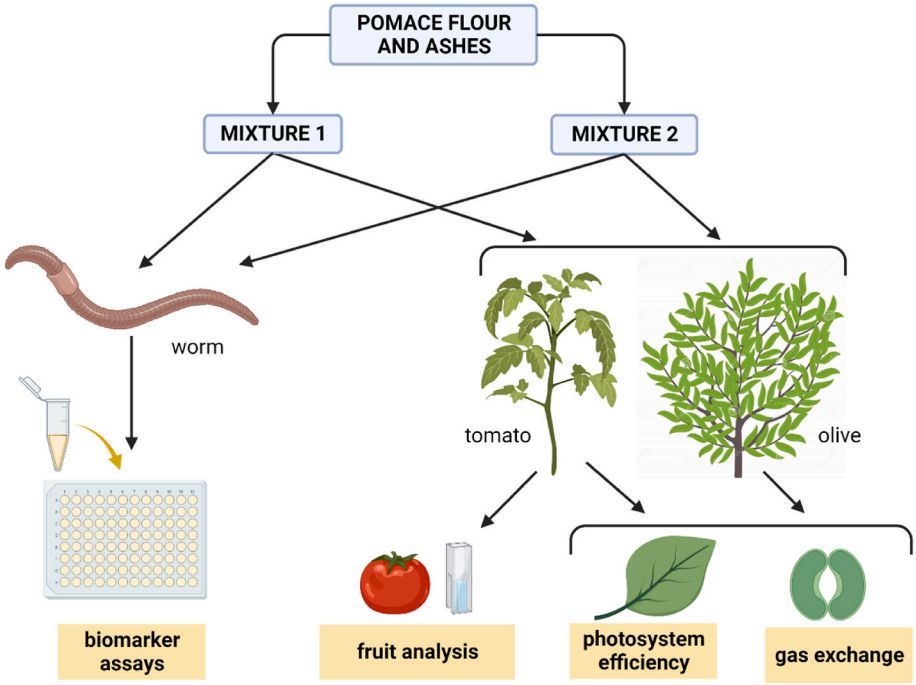

Fig. 1. Experimental plan. Mixtures 1 and 2 from pomace flours and ashes were tested for potential toxicological activity by determining the biomarker response of worms. In addition, Mixture 1 and Mixture 2 were also used as amendments for the cultivation of tomato and olive plants. The effects of both mixtures were evaluated by analyzing photosynthetic parameters; in the case of tomato, we also analyzed the effects on fruit quality.

Table 1
Quantity of soil, quantity of powder, humidity, pH and conducibility of the experimental groups for testing the sublethal effects of Mix 1 (50% de-oiled pomace flour, 50% kernel ash) and Mix 2 (70% de-oiled pomace flour, 30% kernel ash) on *E. fetida*.

Treatment	Quantity of soil	Quantity of powder	Humidity (%)	pH	Conducibility (μS)
Control	1000 g	0 g	40	9.30	12
Mix1	960 g	40 g	40	9.64	13
	980 g	20 g	40	9.64	13
Mix2	960 g	40 g	40	9.51	12
	980 g	20 g	40	9.51	12

homogenate supernatant and 250 μL of a 0.2 mM NADH (SIGMA N-8129) solution prepared in Tris–NaCl buffer (0.1 M, pH 7.2). 4 wells loaded with 40 μL of Tris–NaCl buffer (0.1 M, pH 7.2) and 250 μL of the NADH solution were used as assay blanks. A Multiskan SkyHigh Thermo Scientific microplate reader was used to measure absorbance at a wavelength of 340 nm immediately after the addition of 40 μL of ice-cold 10 mM pyruvate (SIGMA P-2256) in Tris–NaCl buffer (0.1 M, pH 7.2), and after 5 min of incubation. Enzyme activity was calculated from the slope of the absorbance curve and expressed as μmol of substrate hydrolyzed/min. The lysis of *Micrococcus lysodeikticus* was measured using the standard turbidity assay described by Keller et al. (2006) with a slight modification. For each sample, 25 μL PMS was added in quadruplicate to the plate, and 175 μL/well of *M. lysodeikticus* in 0.1 M phosphate buffer (pH 5.9) was quickly added to three sample wells and each of the standard wells. The fourth well containing PMS received a 175 μL phosphate buffer and served as a blank. Plates were assessed for absorbance at 450 nm with a spectrophotometer (Microplate Reader Model 550, BioRad) immediately (T_0) and again after 5 min (T_5). The result was expressed in HEL concentration (μg/μL) via linear regression of the standard curve. Protein concentrations were measured spectrophotometrically using the BioRad Protein Assay (BioRad) according to the Bradford method (Bradford, 1976).

2.3. Plants growth conditions and treatments

Tomato seeds (*Solanum lycopersicum* L., cv. San Marzano nano) were germinated in Petri dishes on filter paper soaked in distilled water in the dark at a constant temperature of 25 °C. The seedlings were then planted in 8,5 L volume pots. Olive trees (*Olea europaea* L., cv. Frantoio) of 18 months of age were taken from the nursery of "Società Pesciatina di Orticoltura" (Pescia, PT, Italy) and transferred to 12 L volume pots. The organic soil used was "Vigor Plant soil" (Vigor plant Italia srl Fombio, Fombio, Italy). The tomato and olive plants were grown in the Botanical Garden of the University of Siena (Italy). During the two months of the experiment, the mean temperature was 25 ± 6.7 °C, with a maximum of 39.2 °C and a minimum of 15.8 °C. There were 4 days with rainfall >1 mm. The trend of temperature and precipitation over the 60 days of the experiment is shown in Fig. S1. Each pot was irrigated manually with 300 mL of water three times per week (tomato) and 500 mL of water twice per week (olive).

A preliminary trial (T_{powder}) was carried out to identify any critical issues caused by a large amount of pomace flour and pomace ash in the soil. Pomace flour and pomace ash were used in powder for the T_{powder} experiment. In order to achieve a ratio of 1:2 (v:v, powder: organic soil), dust equal to 1/3 of the total volume of the pot was added to both the olive and tomato plants.

In the T_{powder} test, plants were divided into three groups (n = 10 for olive trees, n = 6 for tomato plants): one as a control (CTRL), another (Mix 1) with powder added from Mix 1, and the last (Mix 2) with powder added from Mix 2.

A second trial (T_{pellet}) aimed to investigate the effects of the two mixtures in pellet formulation on the physiology of olive and tomato plants, as well as on tomato plant productivity. In the T_{pellet} test, olive trees were divided into three groups (n = 10) and tomato plants into five groups (n = 6). As shown in Table 2, each group received a different amount of pellets of Mix 1 and Mix 2.

Table 2
Description of the study groups involved in the soil improver pellet experiment. The prefix O- indicates tests carried out on olive plants, while the prefix T-indicates tests carried out on tomato plants. For each species (olive and tomato), pellets of Mix 1 (50% de-oiled pomace flour, 50% kernel ash) and Mix 2 (70% de-oiled pomace flour, 30% kernel ash) were tested in the quantities indicated in the "pellet quantity" column.

Treatment	Species	N. individuals	Pellet quantity
O-CTRL	Olive	10	0 g
O-Mix1	Olive	10	60 g of Mix 1
O-Mix2	Olive	10	60 g of Mix 2
T-CTRL	Tomato	6	0 g
T-Mix1 (10 g)	Tomato	6	10 g of Mix 1
T-Mix1 (20 g)	Tomato	6	20 g of Mix 1
T-Mix2 (10 g)	Tomato	6	10 g of Mix 2
T-Mix2 (20 g)	Tomato	6	20 g of Mix 2

2.4. Measurement of soil pH

Soil pH was determined according to the Ministerial Decree n.248 of October 21, 1999 (https://www.gazzettaufficiale.it/eli/gu/1999/10/21/248/so/185/sg/pdf#page=33, last access August 29, 2023). Soil samples were collected in triplicate 30 days after the treatment.

2.5. Determination of photosynthetic efficiency (Fv/Fm) and performance index (PI)

Fv/Fm and PI of mature non-senescent leaves were measured using a portable fluorometer (HANDY-PEA, 2000; Hansatech Instruments, King's Lynn, Norfolk, UK). After 30 min of dark adaptation, leaves were illuminated for 1 s with a light beam (peak at 650 nm, 3000 mol m-2 s-1) and the chlorophyll fluorescence signal emitted by the leaves was recorded. The fluorimeter calculated the maximum quantum efficiency of PSII (Fv/Fm) automatically (Equation (1)):

$$Fv/Fm = (Fm - F0) / Fm \quad (1)$$

Where Fm is the maximum fluorescence value, F0 is the fluorescence of chlorophyll in the leaf sample, and Fv is the difference between Fm and F0. Fv/Fm is a value between 0 and 1, with 1 corresponding to photosystem II's maximum photochemical efficiency, i.e. the condition in which all light energy is converted into chemical energy. PI is a multiparametric expression that evaluates functional variations throughout the photosynthetic apparatus and is calculated as follows (Equation (2)):

$$PI = 1- (F0/Fm) / M0/Vj * (Fm - F0)/F0 é (1-Vj)/Vj \quad (2)$$

F0 and Fm have already been described, while Vj is relative to Fv and M0 is the fluorescence kinetics' initial slope. Fv/Fm and PI were measured in six leaves from each group before the treatment (day 0), one month (day 30) and two months (day 60) after the treatment, during both the T_{pellet} and T_{powder} experiments. The average and standard errors were then computed.

2.6. Leaf gas exchange: stomatal conductance and CO2 Net Assimilation Rate

Leaf gas exchange analysis was performed using the LI-6800 Portable Photosynthesis System (LI-COR Inc., Lincoln, NE, USA) equipped with a 6800-01 A chamber with a 2 cm^2 aperture insert. The CO_2 reference concentration was set to 400 ppm, the humidity was set to 60%, and the light beam was set to a saturating level (1600 mol s^{-1}) (Diaz-Espejo et al., 2007). For each group, measurements were taken in the morning (8/9.30 a.m.) on six mature non-senescent leaves. CO_2 Net Assimilation Rate and Stomatal Conductance were measured before the treatment (day 0), one month (day 30) and two months (day 60) after the treatment, during both the T_{pellet} and T_{powder} experiments. Finally, average and standard errors were calculated.

2.7. Number and weight of tomato fruits

In the T_{pellet} experiment, the total number of fruits produced and their total weight were recorded for tomato plants. To estimate the total yield (kg/group and number of fruits/group), the ripe red tomatoes harvested from each group throughout the season were weighed and counted. For each group, the total number and weight of the harvest were calculated.

2.8. Antioxidants extraction for colorimetric analysis

Sample extraction was performed according to the method described by Conti et al. (2019). Briefly, 1 g of tomato pulp was suspended in 3 mL of 70% acetone, homogenized for 5 min, and sonicated for approximately 20 min. The mixture was centrifuged at 1500 g for 5 min, and the supernatants were collected and used to calculate the antioxidant power and phenolic content. The extraction was performed on a pool of ripe red tomatoes collected during the T_{pellet} experiment, before the first month of treatment (day 0/30), between 30 and 40 days of treatment (day 30/40), between 40 and 50 days of treatment (day 40/50), and between 50 and 60 days of treatments (day 50/60). Results are expressed as the mean of the three replicates ± standard error.

2.9. Determination of antioxidant power

FRAP method (Ferric Ion Reducing Antioxidant Power) (Benzie and Strain, 1996) was carried out to determine antioxidant power. For each sample, 20 μL of extract was mixed with 2040 μL of 300 mM acetate buffer pH 3.6, 200 μL of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine), and 200 μL of 20 mM $FeCl_3$. After 1 h-incubation at 37 °C, the absorbance of samples was measured using a UV–Vis spectrophotometer (wavelength set at 563 nm). The absorbance values were interpolated on a standard curve using known ferrous sulfate solutions. The antioxidant power of each group was expressed in mmol of ferrous chloride equivalent per 100 g of matter.

2.10. Determination of phenolic content

The total polyphenols content was determined by the Folin-Ciocalteu colorimetric assay (Singleton and Rossi, 1965). Briefly, 500 μL of extract was mixed with 3950 μL of distilled water, 250 μL of Folin-Ciocalteu reagent (Sigma Chemical, St. Louis, Missouri, USA), and 750 μL of a sodium carbonate saturated solution (Na_2CO_3) for each reaction. After a 30-min incubation at 37 °C, the absorbance of each sample was measured at 795 nm using a UV–Vis spectrophotometer. The absorbance value was interpolated using a standard curve of a known gallic acid solution (Sigma Chemical, St. Louis, Missouri, USA). Total phenolic content was measured in milligrams of gallic acid equivalents (GAE) per 100 g of matter.

2.11. Statistical analysis

The Shapiro-Wilk test was used to assess whether the data distribution was parametric or non-parametric. As the data did not follow a normal distribution, the non-parametric Kruskal-Wallis rank test for population equality was used to assess statistically significant differences between treatment groups. Accordingly, for each biomarker, the null hypothesis that the median was equal in the control and treatment populations was tested. When the difference between the medians was found to be significant, Dunn's multiple pairwise comparison tests were performed using the Benjamini-Hochberg adjustment (BH step-up procedure). For plant physio-chemical data, Dunn's multiple pairwise comparison was used to assess the difference between treatments and between treatments and control within the same time point. The assumed level of significance was 0.05, and all statistical analyses were performed using R studio (2023.06.0 build 421). Graphs were generated

using Microsoft Excel software.

3. Results and discussion

3.1. Chemical profile of the mixtures

The chemical profiles of Mix 1 and 2 are shown in Table 3.

Except for calcium and exchangeable potassium, which are significantly higher in Mix 1 and 2, the mixtures have similar elemental contents and pH values. Compared to other fertilizers, such as the pure ash (9.3 g kg^{-1}; 23.5 g kg^{-1} K) analyzed by Nogales et al. (2006) or the olive pomace composts (Montemurro et al., 2015) (3.26/6.28 g kg^{-1} P; non-detectable K), the amount of total P and K is remarkable. Since most of the nitrogen is in organic form, it is inaccessible to plants. However, the C/N ratio must be taken into account: a ratio lower than 25 favors microbial activity, resulting in the formation of mineral nitrogen from organic nitrogen (Diacono and Montemurro, 2010). Although the ratio of the mixtures is lower than the threshold (18.8 for Mix 1 vs. 24.1 for Mix 2), Mix 1 is preferable. The pH of the pomace flour and ash Mix is alkaline, as previously reported (Nogales et al., 2006). The use of pomace ash allows us to solve problems related to the typical acidity of pomace (Ameziane et al., 2019).

The presence of heavy metal residues, organochlorine pesticides (OC) and polycyclic aromatic hydrocarbons (PAH), were reported in Table 3. Heavy metals are all below the legal limits stated in the Italian Legislative Decree 75/2010 regarding the chemical composition of compost and amendments. Polycyclic aromatic hydrocarbons are below the limit of instrumental detectability for all mixtures, and organochlorines (total PCBs and total DDT) align with the values reported for most soils used for agricultural purposes.

Table 3
Chemical profile, Heavy metals, organochlorine pesticides (OC) and polycyclic aromatic hydrocarbons (PAH) of Mix 1 (50% kernel ash; 50% de-oiled pomace flour) and of Mix 2 (70% kernel ash; 30% de-oiled pomace flour).

Parameter	u.m.	Mix 1	Mix 2	LOD	Legal limits Italian D.M. 46/2019
pH	units	10.3	10.9	–	
Dry residue	%	94.6	95.8	0.1	
Organic Carbon	g/kg	228.9	228.4	0.1	
Organic Nitrogen	g/kg	11.77	9.05	0.01	
Total Nitrogen	g/kg	12.19	9.46	0.01	
Total Phosphorous	mg/kg	20,442	18,558	1	
Potassium	mg/kg	93,516	90,557	1	
Assimilable Phosphorous (P_2O_5)	mg/kg	542	204	1	
Exchangeable Potassium	mg/kg	4944	15,074	1	
Exchangeable Calcium	mg/kg	16,595	5134	1	
Pb	mg/kg	3	2.5	1	140
Cd	mg/kg	0.2	0.1	0.1	1.5
Ni	mg/kg	19.4	18.1	0.6	100
Zn	mg/kg	64.7	64.4	0.6	500
Cu	mg/kg	183.5	200.8	0.6	230
Hg	mg/kg	<0.1	<0.1	0.1	1.5
$Cr0_3$	mg/kg	<0.2	<0.2	0.2	0.5
Cr	mg/kg	20.5	19.5	0.6	
As	mg/kg	2.6	2	1	
PAHs	mg/kg	<0.01	<0.01	0.01	0.1
PCBs	µg/kg	14.67	11.3	0.1	60
DDTs	µg/kg	13.52	7.93	0.1	10

3.2. Toxicological effect on soil organisms

Earthworm survival rate and biomass are reported in Table S1. The following section shows the biomarker responses of 44 samples of *Eisenia fetida* exposed to different amounts and concentrations of dry pomace and ash. The potential toxicological effects were evaluated using biomarkers of oxidative stress (LPO, GST), energy metabolism (LDH), and immune system (LYS). The mean, standard deviation, and number of results are reported in Table S1.

Fig. 2A shows the LPO levels measured on *Eisenia fetida* exposed to different pomace flour and ash mixtures. The lipid membrane peroxidation levels were not statistically different from the control. Our results were lower than those of Campani et al. (2017), who measured LPO levels in *Eisenia fetida* samples exposed to olive mill waste and found no significant difference from the control. Kovačević et al. (2022) reported higher levels of LPO in *Folsomia candida* exposed to soil contaminated with OMW. The increased MDA level indicates that the antioxidant system was compromised and cell membranes were damaged. The authors hypothesized that the presence of polyphenols, which weaken the antioxidant system of bioindicator organisms, was the cause of oxidative stress. The absence of LPO in all the treatments indicates that the matrices did not induce oxidative stress in *E. fetida*.

Fig. 2B shows the results related to the activity of the GST enzyme measured in samples of *Eisenia fetida* exposed to different concentrations of pomace flour and ash. The GST activity levels did not change significantly, although the mean values were higher than the control in all treatments. GST is an important phase II metabolic enzyme in organisms, as it catalyzes the conjugation of the thiol group of reduced glutathione to xenobiotic and electrophile compounds (Trigui et al., 2022). Some authors describe the depletion of GST due to the activation of the detoxification mechanism in organisms exposed to olive mill waste (Hackenberger et al., 2018; Trigui et al., 2022). Other authors have found the induction of GST activity in earthworms exposed to soil treated with wastewater, indicating the activation of GST when the organism is exposed to organic compounds, trace elements, and pathogens (Mkhinini et al., 2019; Velki and Hackenberger, 2013). The lack of change in all the treatments indicates that the matrices did not contain substances that could induce or deplete the glutathione S-transferase enzyme.

Fig. 2C shows the results relating to the activity of the LDH enzyme measured in specimens of *Eisenia fetida* exposed to different concentrations of pomace flour and ashes. LDH activity did not undergo significant changes compared to the control in any treatment. The study of Rico et al. (2016), reports a decrease in LDH activity levels exposed to the pesticide Prochloraz (71, 59, 48, 28, 30 nmol x min-1 x mg-1 in concentrations of 0, 188, 216, 249, 286 mg/kg d.w. respectively), while the pesticide Tebuconazole caused an increase in LDH activity at all concentrations except the highest, where the enzyme activity dropped sharply (69, 79, 80, 98, 58 nmol x min-1 x mg-1 at concentrations of 0, 63, 95, 142, 213 mg/kg d. w., respectively). Glyphosate (GBH), as shown in the study by Owagboriaye et al. (2021) on the species *Libyodrilus violaceus*, caused an alteration in LDH levels with statistically significant differences between all treatment groups and the control. As a result, the LDH levels obtained in both of the aforementioned case studies are higher than the results of the current study.

Fig. 2D shows the results of the LYS activity measured in *Eisenia fetida* samples exposed to different concentrations of pomace flour and ash. The graph shows that the LYS activity showed statistically significant differences (K–W: H 10.55; $p = 0.005091$) between Mix 2 (40 g) with respect to Mix 1 (20 g) (Dunn's test $p = 0.00183$), Mix 1 (40 g) and the control (Dunn's test $p = 0.0352$), with lower values in all the

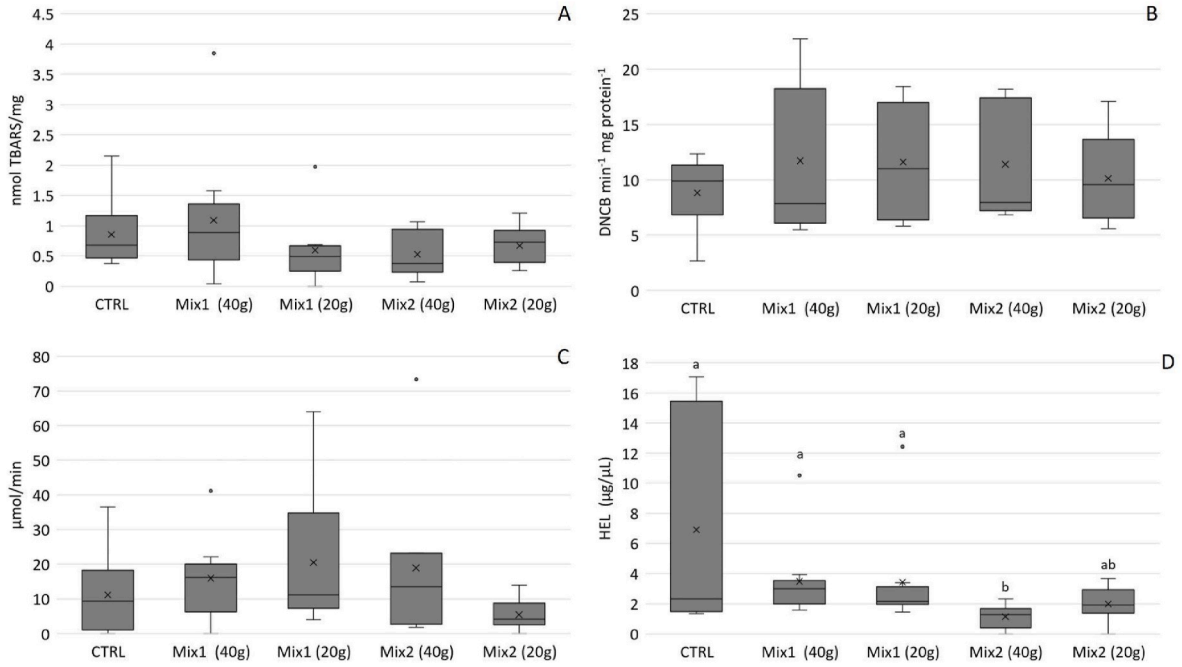

Fig. 2. Boxplots of (A) the lipid peroxidation levels LPO, (B) Glutathione S-transferase activity GST, (C) lactate dehydrogenase activity LDH and (D) the lysozyme activity LYS measured in *E. fetida* control (CTRL) and exposed to different amounts of Mix 1 (50% kernel ash; 50% de-oiled pomace flour) and of Mix 2 (70% kernel ash; 30% de-oiled pomace flour). Different letters denote statistical differences (p-value <0.05) between the groups according to Dunn's test.

treatments compared to the control. As highlighted in the study by Gautam et al. (2020), a decrease in LYS activity can be induced by heavy metals. The earthworm *Metaphire posthuma,* studied in the above work, suffered a decrease in LYS levels after exposure to soil contaminated with tannery effluents containing cadmium, chromium, lead, and mercury.

The tests described above indicate that the matrices with different pomace flour and ash mixtures had no sublethal effects on *E. fetida* during long-term exposure. This evidence would indicate a low-risk use of these matrices on soil.

3.3. General considerations about mixture's effects on olive and tomato plants

First, we tested for potential adverse effects of the compounds on tomato and olive crops using an excess amount of the soil amendment in powder form (T_{powder}). We found that olive trees showed no damage to physiological parameters. On the other hand, tomato plants in the T_{powder} test died prematurely, most likely due to the hygroscopic properties of the powder as well as the excess nutrients it contains, which put more pressure on herbaceous and seasonal plants like tomatoes than on olive trees. Therefore, the results of T_{powder} are limited to olive trees.

3.4. Soil pH

The soil pH measured at day30 showed statistically significant differences between the experimental groups (K–W: H = 27.938, *p-value* = 0.001847). In the T_{powder} experiment, the alkalinity of mixtures is maintained in the soil, and both O-Mix1 (*p-value* = 0.00024) and O-Mix2 (*p-value* = 0.00099), the two treated groups, showed a statistical difference compared to the control (Table 4). The pellet formulation eliminates the pH difference between control and treated groups: no significant differences were found in olive trees during the T_{pellet}. Regarding the T_{pellet} of tomato plants, all treated samples differ from the control (*p-value* < 0.05), except for the treatment with 10 g of pellet of Mix 1 (*p-value* = 0.190,520). This was most likely due to the smaller volume of pots. Even without additives, the soil is slightly alkaline, and this is most likely due to the irrigation water, which had a pH of 7.6.

Table 4
Soil pH measured at day30 in control and treated samples of the analyzed species. In the "Sample" column, the prefix O indicates tests carried out on olive plants, while the prefix T indicates tests carried out on tomato plants. Different letters denote statistical differences (p-value <0.05) between the groups according to Dunn's test.

Trial	Sample	Soil pH
T_{powder}	O-CTRL	7.9 ± 0.1^{b}
	O-Mix 1	9.8^{a}
	O-Mix 2	9.5 ± 0.1^{a}
T_{pellet}	O-CTRL	8.1 ± 0.1^{b}
	O-Mix 1	8.6 ± 0.1^{b}
	O-Mix 2	8.5 ± 0.4^{b}
	T-CTRL	7.9 ± 0.2^{b}
	T-Mix 1 (10 g)	8.4 ± 0.5^{ab}
	T-Mix 1 (20 g)	8.7 ± 0.1^{a}
	T-Mix 2 (10 g)	8.8 ± 0.2^{a}
	T-Mix 2 (20 g)	9.1 ± 0.4^{a}

3.5. Effects of mixtures on photosynthesis of olive and tomato plants

The value A in the photosynthesis analysis is a parameter that represents the net CO_2 assimilation rate in the leaves. When olive plants were subjected to treatment with the powder mixtures (Fig. 3A), we observed a common trend for both control and treated plants. At the beginning of the treatment, the A value was found to be 20–30 µmol m^{-2} s^{-1}, but after 30 days of treatment, the values decreased drastically in all plants to about 10 µmol m^{-2} s^{-1}. The decrease was greater in the O-Mix2 group, but no differences were found compared to the control. After 60 days of treatment, the A values increased slightly in all groups but did not return to the initial values. The treated group O-Mix1 was significantly different from the control (K–W: H = 6.7661, *p-value* = 0.03394, Dunn's test *p-value* = 0.032508).

The value gs measures the stomatal conductance of a leaf, which estimates the rate of transpiration through the leaf stomata. Since gs is

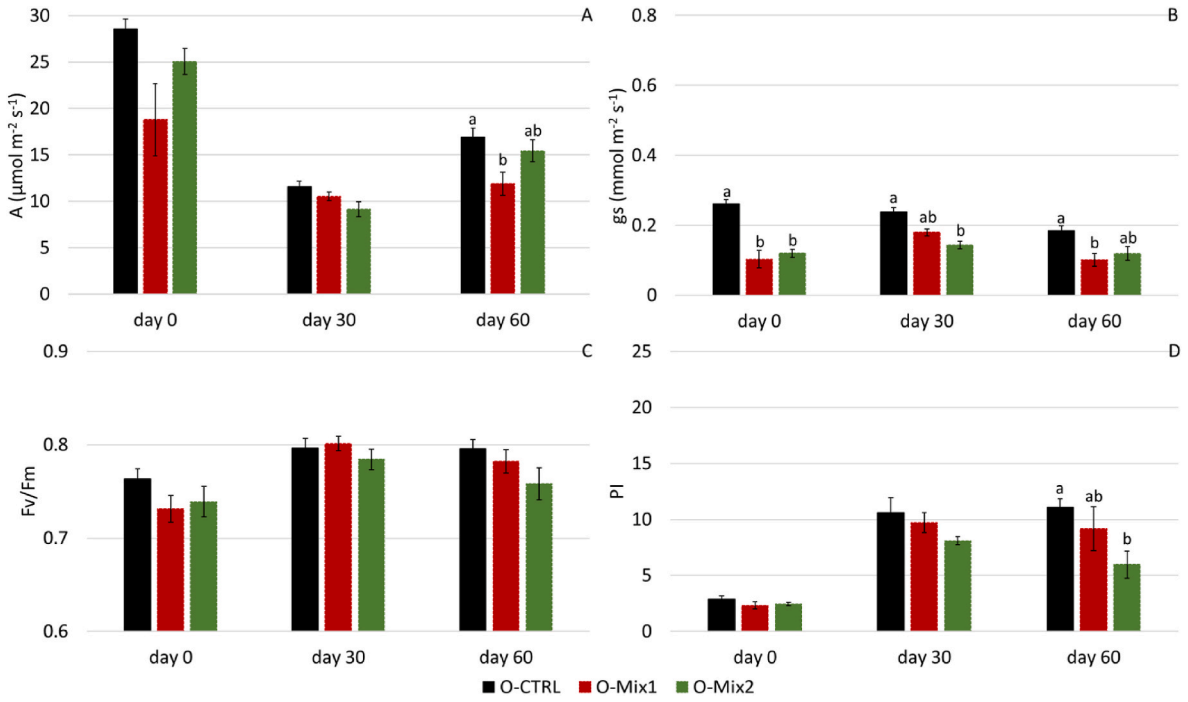

Fig. 3. Analysis of the main photosynthetic parameters in olive trees (indicated by the prefix O-) measured at day 0, day 30, and day 60 during the T_{powder} experiment. The bars represent mean ± standard error. Values for control are in black, values for plants treated with Mix 1 (50% de-oiled pomace flour, 50% kernel ash) are red, and values for plants treated with Mix 2 (70% de-oiled pomace flour, 30% kernel ash) are green. (A) Measurements of net carbon assimilation rate (A); (B) Analysis of stomatal conductance (gs); (C) Analysis of photosynthetic efficiency (Fv/Fm); (D) Measurements of performance index (PI). For each time point, different letters denote statistical significance (p-value <0.05) according to Dunn's test.

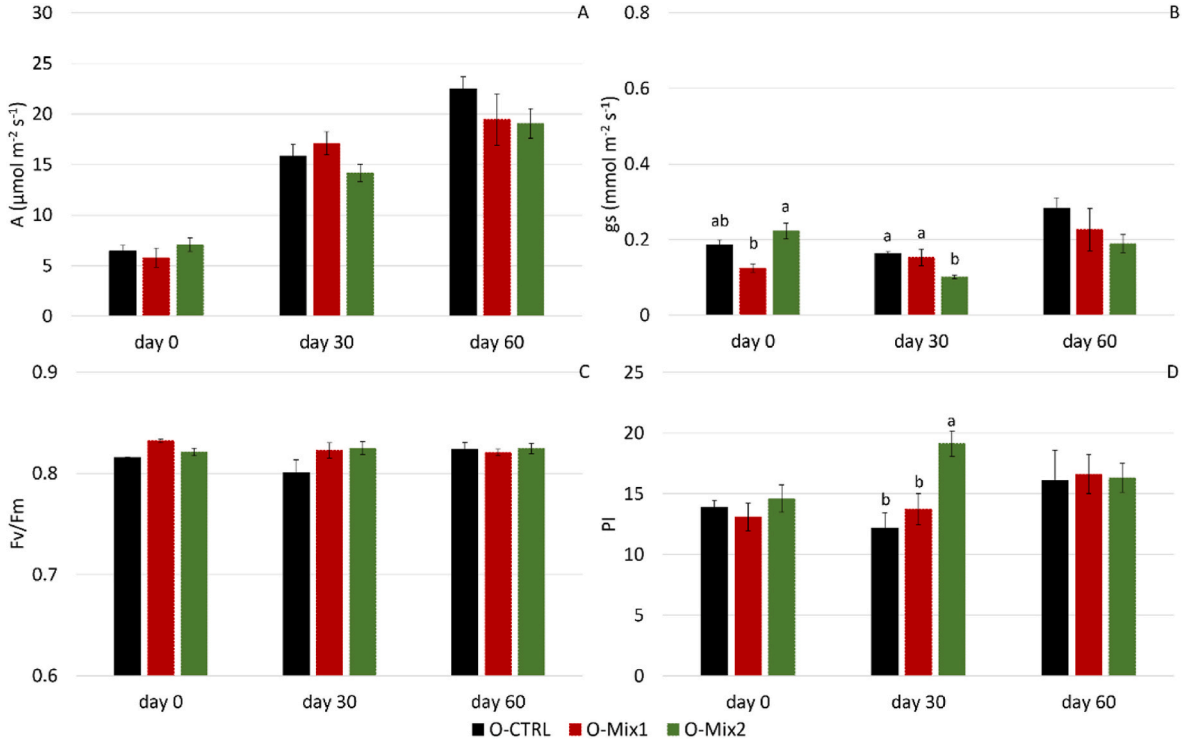

Fig. 4. Analysis of the main photosynthetic parameters in olive trees (indicated by the prefix O-) measured at day 0, day 30, and day 60 during the T_{pellet} experiment. The bars represent mean ± standard error. Values for control are in black, values for plants treated with Mix 1 (50% de-oiled pomace flour, 50% kernel ash) are red, and values for plants treated with Mix 2 (70% de-oiled pomace flour, 30% kernel ash) are green. (A) Measurements of net carbon assimilation rate (A); (B) Analysis of stomatal conductance (gs); (C) Analysis of photosynthetic efficiency (Fv/Fm); (D) Measurements of performance index (PI). For each time point, different letters denote statistical significance (p-value <0.05) according to Dunn's test.

influenced by the degree of stomatal aperture, it affects the photosynthesis rate, as it determines the availability of CO_2. At day 0 (K–W: H = 13.455, *p-value* = 0.001198), stomatal conductance was unexpectedly higher and significantly different in the control samples compared to the O-Mix1 (Dunn's test *p-value* = 0.001050) and O-Mix2 (Dunn's test *p-value* = 0.018722) treated groups (Fig. 3B). The difference between the control and O-Mix2 was also found on day 30 (K–W: H = 14.39, p-value = 0.000751, Dunn's test *p-value* = 0.000451) and between control and O-Mix1 on day 60 (K–W: H = 6.504, *p-value* = 0.0387, Dunn's test *p-value* = 0.041686).

In photosynthesis analysis, Fv/Fm is commonly used to monitor the efficiency of the photosynthetic apparatus under different environmental conditions (Parri et al., 2023; Piccini et al., 2020), as it drops below 0.800 when plant stress occurs (Björkman and Demmig, 1987). As shown in Fig. 3C, the parameter did not change consistently during the treatment and it showed no significant differences between control and treated plants. PI is the performance index, which is a composite parameter that takes into account the absorption, capture, and conversion efficiencies of PSII. The PI parameter (Fig. 3D) showed some more pronounced differences among the samples. After 30 days of treatment, O-Mix1 and O-Mix2 showed a lower PI than the control. After two months of treatment, the difference between O-Mix2 and the control group was significant (K–W: H = 4.9054, *p-value* = 0.04606, Dunn's test *p-value* = 0.032543).

The analysis of Mix 1 and Mix 2 applied to olive plants in pellet form showed no significant differences between control and treated plants. Photosynthesis, as measured by the A-value (Fig. 4A), was practically the same for both control plants and plants treated with Mix 1 and Mix 2, and no significant differences were observed during the experiment. Stomatal conductance (Fig. 4B) showed some differences on day 0: O-Mix1 was lower and significantly different compared to O-Mix2 (K–W: H = 7.7077, *p-value* = 0.0212, Dunn's test *p-value* = 0.017593). At day30 (K–W: H = 7.4491, p-value = 0.02412), gs in O-Mix2 decreased and the value significantly differed from the control (Dunn's test p-value = 0.042177) and from O-Mix1 (Dunn's test p-value = 0.035798). However, after two months of treatment, gs in the treated groups was comparable to that one of the control. As shown in Fig. 4C, Fv/Fm was always maintained above 0.8 in all groups, indicating good plant health (Björkman and Demmig, 1987). PI (Fig. 4D) showed an increase in plants treated with Mix 2 in the middle of the treatment (K–W: H = 7.7308, *p-value* = 0.02095) and O-Mix2 was significantly different from O-Mix1 (Dunn's test *p-value* = 0.059210) and O-CTRL (Dunn's test *p-value* = 0.024321). However, at the end of the experiment, the values of all the plants were practically identical.

Tomato plants were analyzed after the addition of Mix 1 and Mix 2 in pellet form at two different doses (10 g and 20 g). The net carbon assimilation rate (Fig. 5A) increased in all groups from the beginning to the end of the experiment. This could be due to the progression of the growing season. Interestingly, tomato plants treated with both doses of Mix 2 showed a higher net carbon assimilation rate after one month of treatment (K–W: H = 11.07, *p-value* = 0.02578) and this difference was maintained at the end of the treatment (K–W: H = 14.954, *p-value* = 0.004797). In particular, at day30, T-Mix2 10 g and T-Mix2 20 g resulted significantly different from T-CTRL (Dunn's test *p-value* = 0.019800 and 0.008869, respectively) and from T-Mix1 10 g (Dunn's test *p-value* = 0.044568 and 0.021695). At day 60, T-Mix2 10 g significantly differed from T-CTRL (Dunn's test *p-value* = 0.010149), T-Mix1 10 g (Dunn's test *p-value* = 0.001245), and T-Mix1 20 g (Dunn's test *p-value* = 0.008526); T-Mix2 20 g resulted significantly different from T-Mix1 10 g (Dunn's test *p-value* = 0.016786). This effect was similar for the measure of stomatal conductance (Fig. 5B). Again, at day 30, control plants showed gs values comparable to those of the plants treated with Mix 1, both

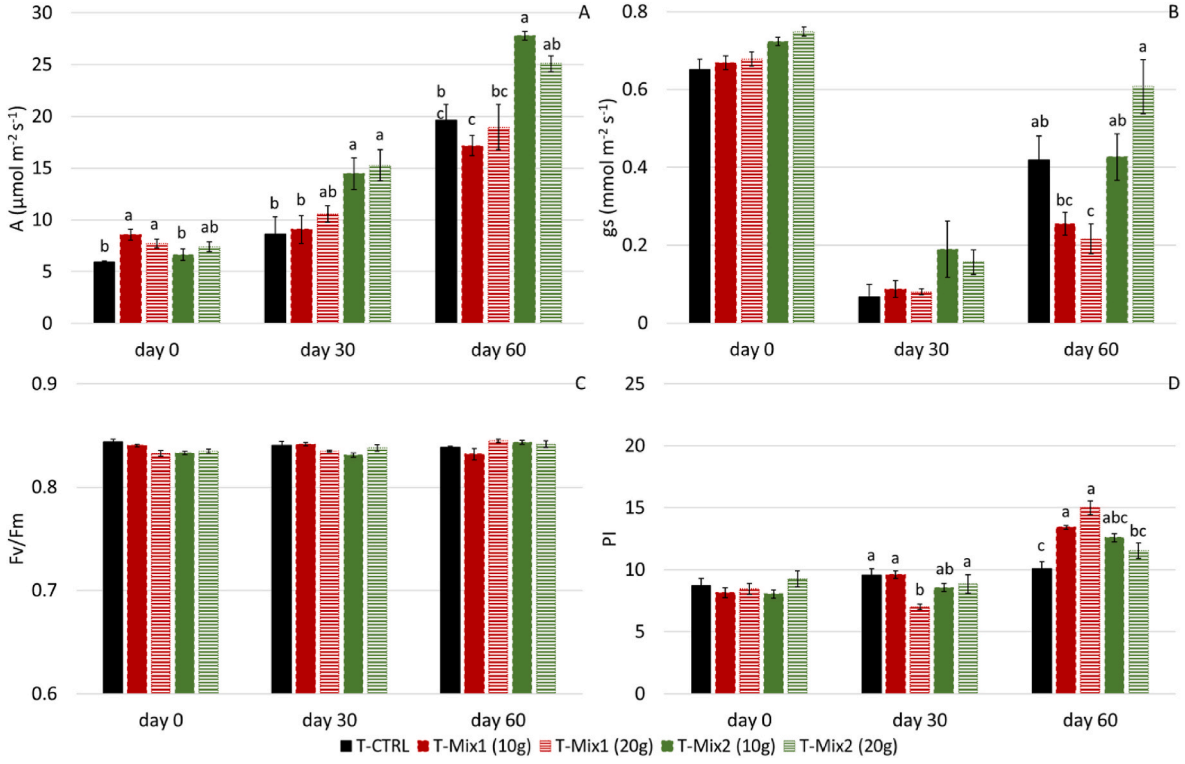

Fig. 5. Analysis of the main photosynthetic parameters in tomato plants (indicated by the prefix T-) measured at day 0, day 30, and day 60 during the T_{pellet} experiment. The bars represent mean ± standard error. Values for control are in black, values for plants treated with Mix 1 (50% de-oiled pomace flour, 50% kernel ash) are red (full bars for lower the dose, striped bars for higher dose), values for plants treated with Mix 2 (70% de-oiled pomace flour, 30% kernel ash) are green (full bars for lower dose, lines bars for higher doses). (A) Measurements of net carbon assimilation rate (A); (B) Analysis of stomatal conductance (gs); (C) Analysis of photosynthetic efficiency (Fv/Fm); (D) Measurements of performance index (PI). For each time point, different letters denote statistical significance (*p*-value <0.05) according to Dunn's test.

concentrations, but lower than those of plants treated with Mix 2 (both concentrations). At day 60 (K–W: H = 14.214, p-value = 0.006642), T-Mix2 20 g was found to be higher and significantly different from O-Mix1 10 g and O-Mix1 20 g (Dunn's test p-value = 0.004973 and 0.001538, respectively). T-Mix2 10 g resulted higher and significantly different from T-Mix1 20 g (Dunn's test p-value = 0.027024). Fv/Fm data showed no differences between control and treated tomato plants. As in the olive trees, the Fv/Fm values (Fig. 5C) fluctuated very little from day 0 to day 60, always remaining within the physiological range. As shown in Fig. 5D, the fluctuations in PI were more pronounced than in Fv/Fm. After the first month of treatment, T-Mix1-20 g had a lower and significantly different PI value compared to the other groups (K–W: H = 11.255, *p-value* = 0.02385). In particular, it was significantly different from T-CTRL (Dunn's test *p-value* = 0.004010), T-Mix1-10 g (Dunn's test *p-value* = 0.002628), and T-Mix2-20 g (Dunn's test *p-value* = 0.03494). The differences increased at the end of the experiment, when all treated groups had a higher PI compared to the other time points. At day 60, T-Mix1-10 g and T-Mix1-20 g were higher and significantly different from the control (K–W: H = 16.126, *p-value* = 0.002854; Dunn's test *p-value* = 0.005958 and 0.000334, respectively).

The de-oiled olive pomace has already been proposed to improve soil management and crop production. For example, the product was tested in a high-density olive orchard, showing that the treatment was non-toxic and that the treated plants behaved similarly to the control plants (Camposeo and Vivaldi, 2011). In the case of wheat crops, the addition of de-oiled two-phase olive mill waste resulted in increased grain yields after a two-year experiment, suggesting that the mixture could be a valuable soil amendment and source of organic matter (López-Piñeiro et al., 2008). These findings suggest that the use of the product in crops has the potential to increase production and improve overall agricultural practices. De-oiled olive pomace can be easily composted while maintaining its nutritional quality. Over the course of several years, a compost preparation was applied to an olive orchard, and the treatment resulted in higher fruit quality and increased availability of basic nutrients and carbon (Fernández-Hernández et al., 2014). Increases in nutrients such as phosphorus, calcium, zinc, and boron were also observed in bean and sunflower crops, although some negative effects on plant growth were also observed (Ilay et al., 2013). Not to mention the anti-weed properties of de-oiled olive pomace, making it an environmentally friendly and alternative product (Russo et al., 2015). These few examples show that de-oiled olive pomace has been used as an amendment and nutrient source in several crops, with mostly positive results, leading to increased nutrient and carbon availability. In our experiment, we found that the application of different doses of both mixtures as pellets to tomato plants and young olive trees had no negative effect on the photosynthetic parameters. On the contrary, the application of both mixtures in powder form had a significant negative effect on plant growth, as mentioned above. The effects were most likely caused by the higher doses of powder compared to the pellet, as well as the fact that pellets release nutrients more slowly. At all doses tested (10 g and 20 g), A, gs, Fv/Fm and PI were comparable between control plants and plants treated with Mix 1 and 2. Some variations in values were reported, but these were considered physiological. For example, tomato plants treated with Mix 2 (20 g) had a slight increase in gs and tomato plants treated with Mix 1 (20 g) had a slight increase in PI. It is not surprising that the addition of nutrient-rich compost material can cause slight changes in physiological parameters. Indeed, it has been reported that basic photosynthetic parameters improved only slightly in olive plants amended with compost or olive mill wastewater (Chehab et al., 2019). These minor changes in physiological parameters can be attributed to the nutrient content present in the compost material (Chehab et al., 2019). These minor changes in physiological parameters can be attributed to the nutrient content present in the compost material.

3.6. Evaluation of tomato fruit quality and yield

According to the higher values of physiological parameters observed in the treated groups compared to the control, differences were observed when considering the number and weight of tomato fruits. This analysis could not be performed for olive plants because we used two-year-old olive plants that had not yet set fruit. The number of fruits was higher in tomato plants treated with Mix 2 (10 g) and also in those treated with Mix 1 (20 g) (Table 5). The other treatments were only slightly higher than the control. As shown in Table 5 the analysis of fruit number was similar to that of fruit weight. Again, T-Mix2 (10 g) had a significantly higher fruit weight compared to all other treatments. In this case, however, all treated plants had a higher fruit weight than the control plants. These results suggest that both Mix 1 (20 g) and Mix 2 (10 g) treatments had a positive effect on the fruit yield of tomato plants. Additionally, it is noteworthy that although all treated plants had higher fruit weights compared to the control, the Mix 2 (10 g) treatment had the highest increase in fruit weight among all treatments.

Fig. 6A and B show the total antioxidant capacity and the polyphenol content of tomato plants, respectively. The results are given from day 30 to day 60, with tomatoes collected in the ten days between two different time points. The first point to note is that the addition of Mix 1 (both doses) and Mix 2 (lower dose) accelerated fruit ripening; in fact, no tomatoes were found from control plants until day 30. This suggests that the addition of Mix 1 and Mix 2 had a significant effect on the ripening process of tomatoes. It also suggests that these mixtures may contain compounds or nutrients that promote faster fruit ripening compared to the control plants. The second factor to consider is that tomato production is more consistent throughout the analysis period. For example, no tomatoes were found on control plants between 40 and 50 days (day 50, Fig. 6A and B), whereas plants supplemented with either Mix 1 or Mix 2 had ripening tomatoes. This suggests that the addition of Mix 1 and Mix 2 not only accelerates the ripening process but also could ensure more reliable and consistent tomato production. These findings highlight the potential of using these mixtures as a practical and effective method to improve tomato cultivation and increase yields. The trends in the two graphs are very similar because polyphenols are the most abundant antioxidants in tomato fruit. Polyphenolic compounds include flavonoids such as rutin, quercetin, naringenin and the most abundant carotenoid, lycopene (Conti et al., 2022). Since no ripening tomatoes were found in the control plants before day 30, the data of T-CTRL at day 0/30 and 40/50 are missing. Until the end of the experiment, there were no significant differences in antioxidant activity between tomatoes from control plants and tomatoes from plants supplemented with either Mix 1 or Mix 2. At day 50/60, T-CTRL showed antioxidant power (Fig. 6A) higher than the other treated groups and significantly different from that of T-Mix1 (10 g) (K–W: H = 11.579, *p-value* = 0.02077; Dunn's test p-value = 0.015466) and T-Mix1 (20 g) (Dunn's test p-value = 0.001620). Same trend for polyphenol content (Fig. 6B), at day 50/60 T-CTRL differed from T-Mix1 (10 g) (K–W: H = 12.915, *p-value* = 0.0117; Dunn's test *p-value* = 0.008057) and T-Mix1 (20 g) (Dunn's test *p-value* = 0.001892). At the beginning of fruit ripening (day 0/30), T-Mix1 (10 g) had a lower and significantly different antioxidant power value compared to T-Mix2 (10 g) (K–W: H = 7.2, *p-value* = 0.02732;

Table 5
Total fruit number and total fruit weight (g) produced during the T_{pellet} experiment by tomato plants control and exposed to different amounts of Mix 1 (50% de-oiled pomace flour, 50% kernel ash) and Mix 2 (70% de-oiled pomace flour, 50% kernel ash).

Sample	Total fruit weight (g)	Total fruit number
T-CTRL	447	18
T-Mix1 (10 g)	762	22
T-Mix1 (20 g)	827	32
T-Mix2 (10 g)	1029	35
T-Mix2 (20 g)	824	21

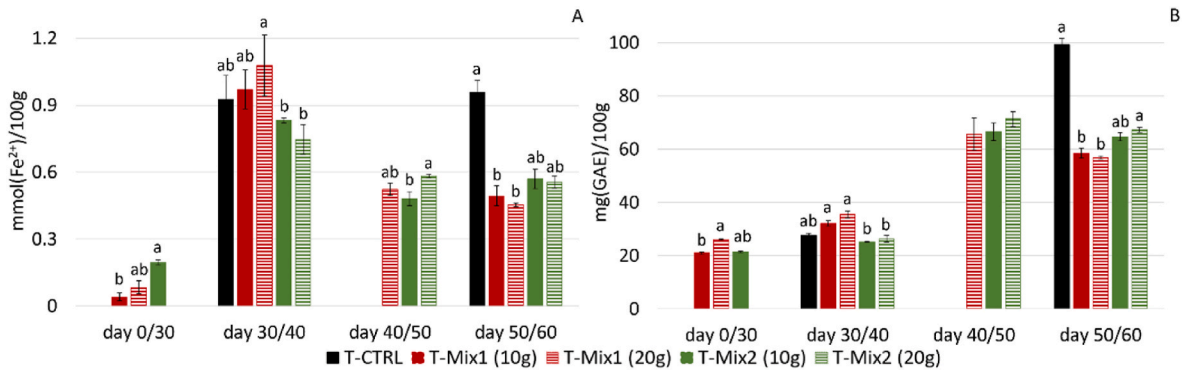

Fig. 6. Analysis of antioxidant power (A) and polyphenols content (B) in tomato fruits collected in the first 30 days of treatment (day 0/30) and every ten days until the end of the T_{pellet} experiment (day 30/40, day 40/50, day 50/60). The bars represent mean ± standard error. Values for control are in black, values for plants treated with Mix 1 (50% de-oiled pomace flour, 50% kernel ash) are red (full bars for lower dose, striped bars for higher dose), values for plants treated with Mix 2 (70% de-oiled pomace flour, 30% kernel ash) are green (full bars for lower dose, striped bars for higher dose). For each time point, different letters denote statistical significance (p-value <0.05) according to Dunn's test.

Dunn's test *p-value* = 0.021871), but the polyphenol content of the two groups was comparable. At day 30/40 (K–W: H = 10.633, *p-value* = 0.03101), T-Mix1 (20 g) showed the highest antioxidant power value. It was significantly different from both T-Mix2 (10 g) (Dunn's test *p-value* = 0.025912) and T-Mix2 (20 g) (Dunn's test *p-value* = 0.022478). These differences suggest that supplementation with Mix 1 and Mix 2 may have differentially affected the antioxidant activity of ripening tomatoes. Further analysis is needed to determine the specific effects of each supplement on tomato ripening and antioxidant activity.

Our results suggest that the application of both mixtures is important in terms of food quality and quantity. The treatment with de-oiled olive pomace not only improved the quality of food production but also had a significant impact on the quantity of food produced, resulting in increased productivity and higher yield of standardized, high-quality food products. Increased fruit size and weight have also been reported for peach trees treated with olive waste and olive mill wastewater as opposed to chemical fertilizers (Atemni et al., 2022). This suggests that not only the amount of food but also the overall growth and development of fruit trees can be improved by using olive waste and olive mill wastewater as a fertilizer. Direct comparisons are difficult due to differences in species and treatment, as well as plant age and chemistry of the product supplied to the plants, but the data suggest that treatment with de-oiled olive pomace pellets can be effectively used as a practical amendment to increase fruit number, size and quality. These results highlight the potential of de-oiled olive pomace pellets as a viable option for fruit production and quality improvement. Further research is needed to explore the specific mechanisms behind these beneficial effects and to optimize application methods for different plant species and growth stages.

4. Conclusion

The challenge of waste management in the oil industry can be addressed by testing the toxicity and usability of de-oiled pomace and pomace ash in agricultural soils. This can create a circular economy between farms, olive mills and olive oil processing plants, reducing reliance on chemical fertilizers and reducing transportation and disposal costs. The study used *Eisenia fetida* as a soil bioindicator to assess the potential toxicological impact of these waste products. The biomarkers showed significant sensitivity, as evidenced by levels of lipid peroxidation, glutathione S-transferase and lactate dehydrogenase that were consistent with those observed in the control group. At the same time, lysozyme activity decreased. Nevertheless, both mixtures were found to be of low toxicity and suitable for use as pellet amendments in crop production. The tomato plant was more sensitive to the treatment compared to the olive tree. The application of the mixtures increased the number and weight of tomato fruits while maintaining the same levels of polyphenols and antioxidants. This means that the mixtures not only improve the development of tomato fruits, but also their nutritional value. In addition, the treatments extended plant productivity throughout the season, resulting in higher overall yields. The potential increase in tomato production and overall productivity can be attributed to the improved physiological resistance of the treated plants. Therefore, the implementation of mixture treatments (such as Mix 2) is a promising solution.

CRediT authorship contribution statement

S. Parri: Conceptualization, Data curation, Investigation, Methodology, Writing - original draft, Writing - review & editing. **T. Campani:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Supervision, Validation, Writing - original draft, Writing - review & editing. **V. Conti:** Data curation, Investigation, Methodology. **G. Cai:** Conceptualization, Writing - original draft, Writing - review & editing. **M. Romi:** Conceptualization, Methodology. **S. Casini:** Conceptualization, Methodology. **R. Zari:** Funding acquisition, Project administration, Supervision. **F. Caldini:** Funding acquisition, Resources, Supervision. **L. Marsili:** Conceptualization, Funding acquisition, Project administration, Resources.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Marsili Letizia reports financial support was provided by Tuscany Region PSR Project Measure 16.2.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at.https://doi.org/10.1016/j.jenvman.2023.119915.

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