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# Phytotoxicity evaluation of nutrient-fortified pomegranate peel powders prepared from food waste and their feasibility as biofertilizers

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## Abstract

Pomegranate peel powder (PPP) is increasingly used as a bioadsorbent to decontaminate wastewaters due to its adsorptive characteristics. The application of nutrient-fortified bioadsorbents as alternatives to chemical fertilizers can provide an innovative and eco-friendly approach for sustainable waste management. Nevertheless, there is extremely limited information regarding their effects on the growth of agricultural crops. We investigated the effects of raw and nutrient-fortified PPPs on oilseed rape (*Brassica napus*). Our results showed that the concentration-dependent in vitro phytotoxicity of high PPP doses (germination indices were 109.6%, 63.9%, and 8.9% at the applied concentrations of 0.05%, 0.5%, and 5%) was diminished by the application of nutrient-fortified PPPs (germination indices were 66.0–83.4% even at the highest doses). In pot experiments, most PPP treatments (especially Raw-PPP and the mixture of N- and P-fortified PPPs) promoted the development of aboveground plant parts. Reorganization of the pattern of protein tyrosine nitration in the root tissues indicated that the plants were acclimated to the presence of PPPs, and thus, PPP treatment induced no or low-level stress. Our findings confirmed that several doses of PPP supplementation were beneficial for the model crop plant when applied in soil. We anticipate that our study will be a foundation for future investigations involving more plant species and soil types, which can contribute to the introduction of nutrient-fortified PPPs as sustainable biofertilizers.

**Keywords** Waste valorization · Sustainability · Bioadsorbent · *Brassica napus* · Soil · Plant morphology

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## 1 Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), approximately 1.3 billion tons of food waste is generated each year globally, of which more than a third (around 39%) is attributed to the European Union (Brito et al., 2022; Roukas & Kotzekidou, 2020). The major food losses can be derived from the production of fruits and vegetables or the supply chain between producers and traders (e.g., harvesting, handling, transportation, storage, and packaging), making the agri-food industry primarily responsible for the production of food waste (Brito et al., 2022). Food waste is rich in C (and often in N) and, thus, can be considered as a resource to produce biofuels or valuable secondary products (Kharola et al., 2022; Singh et al., 2022) via various waste valorization methods: (a) biomethane from anaerobic digestion (Maroušek et al., 2020); (b) bioethanol from fermentation of carbohydrates (Onyeaka et al., 2022); (c) charcoal from combustion (Mardoyan & Braun, 2015); (d) biohydrogen and algae biodiesel from photo-fermentation (Maroušek et al., 2022a); (e) soil amendments, e.g., compost from composting (Awasthi et al., 2020), silica nanoparticles from coir pith (Maroušek et al., 2022b), and biochar from pyrolysis (Maroušek et al., 2019). As the demand for fruit and vegetable production will continue to increase in the future due to global population explosion and economic development, the resulting amount of food wastes and by-products is also expected to increase (Wadhwa et al., 2016), additionally exacerbating environmental problems (e.g., greenhouse gas emission, water, and soil pollution) that ultimately harm the ecosystem and human health (Roukas & Kotzekidou, 2020).

The pomegranate (*Punica granatum* L.), which originated from the Himalayas and Iran, is a crop plant adapted to various agro-climatic conditions and is predominantly grown in the Mediterranean region (including Spain, Morocco, Tunisia, Egypt, Turkey, and Israel), Southwestern American region, Western Asia, and the Middle East (especially in Iran) (Derakhshan et al., 2018; Singh et al., 2018; Smaoui et al., 2019; El Barnossi et al., 2021; Campos et al. 2022). The growing public interest on its nutritional and medicinal potential has escalated the consumption of pomegranate, resulting in an increase in both the cultivated amounts and the generated food waste (Singh et al., 2018; Smaoui et al., 2019). During fruit processing, pomegranate peel (PP) and seeds (together representing about 60% of the total fruit) are potentially discarded as by-products (Campos et al. 2022). Considering that approximately 1.5 million tons of PP is generated annually (Solangi et al., 2021), the appropriate disposal of waste can become an enormous burden on the producing countries. Nevertheless, PP also contains valuable bioactive phytochemicals, including amino acids, vitamins, and various phenolic compounds (e.g., flavonoids, tannins, phenolic acids, etc.), some of which can exhibit antioxidant and antimicrobial activities, thus attracting the attention of food, pharmaceutical, and cosmetic industries (Abd-Rabbu et al., 2021; Derakhshan et al., 2018; Singh et al., 2018; Smaoui et al., 2019). Although the normal consumption of the pomegranate fruit or its extracts is generally beneficial and does not pose a risk to human health, certain phytochemicals might exert toxic effects at higher consumption rates (Akhtar et al., 2015; Ismail et al., 2012; Singh et al., 2018). Furthermore, PP extracts can present phytotoxicity (Campos et al. 2022).

PP waste is traditionally utilized as animal feed; however, there is an increasing interest on novel PP valorization techniques such as the production of value-added products (e.g., essential oils, pectin, reducing sugars, dietary fibers, and cosmetic and medical products) or the recirculation of PP waste in biorefinery processes (e.g., biogas, bioethanol, and biohydrogen). More recently, PP is also used as a soil amendment or bioadsorbent

(Bellahsen et al., 2021a, 2021b; El Barnossi et al., 2021; Roukas & Kotzekidou, 2020) as it contains a substantial variety of functional groups (e.g., –OH and –COH), ensuring its exceptional ion exchange capacity and general adsorptive characteristics (Bellahsen et al., 2021b). Considering that biomass waste-derived bioadsorbents can be renewable, easily produced, cost-efficient, and sustainable, their application promises encouraging results in the fields of bioremediation, waste management, and water purification (Solangi et al., 2021). Recent studies have demonstrated that pomegranate peel powders (PPPs) can be used to remove ammonium nitrogen (81.8% efficiency with the initial $NH_4$-N concentration of 80 mg/L) (Hodúr et al., 2020) or phosphate phosphorus (90% efficiency with the initial $PO_4$-P concentration of 40 mg/L) (Bellahsen et al., 2021a) from wastewaters. Such treatments not only prevent severe environmental ramifications (e.g., overfertilization of soils and eutrophication of water bodies) but also improve the chemical characteristics of adsorbents. Similar to other nutrient-fortified bioadsorbents prepared from peat (Robalds et al., 2016), wheat straw (Ma et al., 2011; Wang et al., 2016; Xie et al., 2013), spent mushroom (Tuhy et al., 2015), and charred wood (Maroušek & Trakal, 2022), or biogas fermentation residue (Maroušek & Gavurová, 2022), PPP bioadsorbents enriched with N and P might represent desirable and environmentally friendly alternatives to chemical fertilizers due to their potential advantages such as biocompatibility, biodegradability, controlled nutrient release, and soil deliverability (Reddy et al., 2017).

Although a few studies reported that PPP supplementation improved the physicochemical characteristics of soil (Jariwala & Syed, 2016; Motamedi et al., 2016); increased the fresh and dry biomass of sage herb in soil (*Salvia officinalis* L.) (Abd-Rabbu et al., 2021); promoted the growth of okra (*Abelmoschus esculentus* L.) (Dayarathna & Karunarathna, 2021), fenugreek (*Trigonella foenum-graceum* L.), and soil microbes (Mercy et al., 2014); and improved the biomass and grain yield of maize (*Zea mays* L.) when used as a coating material on urea fertilizer (Sabahi et al., 2017), there exists a scarcity of knowledge regarding the agricultural utilization of PPPs. Furthermore, the effects of nutrient-fortified PPP adsorbents on plant development have not yet been investigated. Therefore, we conducted this study to provide valuable new information by investigating the effect of raw and nutrient-fortified PPPs on early plant development in two experimental setups. We hypothesized that nutrient-fortified PPPs exhibit low in vitro phytotoxicity and promote plant growth when used as soil amendments and hence can be introduced as renewable biofertilizers.

## 2 Materials and methods

### 2.1 Preparation of PPPs

PP was collected, cut into small pieces, and washed several times with distilled water to remove impurities. Next, oven-dried PP (105 °C, 2 h) was powdered in a grinder (particle size < 250 µm), resulting in a material referred to as raw PPP (hereafter Raw-PPP) (Bellahsen et al., 2021b). Raw-PPP was further used to prepare N-fortified (N-PPP) and P-fortified PPP (P-PPP) by adsorbing ammonium nitrogen and phosphate, respectively, from milking parlor unit (located near Szeged, Hungary) wastewater according to methods described by Hodúr et al. (2020) and Bellahsen et al. (2021a). The detailed methodology and the characterization of both the wastewater and prepared PPPs have been described in our previous studies (Hodúr et al., 2020; Bellahsen et al., 2021a, 2021b). We also prepared Mix-PPP containing a 1:1 mixture of N-PPP and P-PPP.

### 2.2 Plant material

*Brassica napus* L. (oilseed rape; cv. GK Gabriella) was used as a model plant in all experiments. *Brassica* seeds were provided by Cereal Research Non-Profit Ltd. (Szeged, Hungary). Oilseed rape, also known as rapeseed, has substantial importance both economically and agriculturally because it is the second most cultivated oil crop in the world (Mészáros et al., 2022).

### 2.3 In vitro phytotoxicity tests

Dicotyledonous plants are often more sensitive to environmental stressors than monocotyledonous plants and thus can be considered as better bioindicators (Bari & Kato-Noguchi, 2017; Liwarska-Bizukojc, 2022). Therefore, rapeseed (*B. napus* L. cv. GK Gabriella) was used for investigating the effect of various PPPs on seed germination and early root development of higher plants in this study. For this purpose, we used a combination of methods described by Molnár et al. (2020) and Bodor et al. (2021). Surface-sterilized seeds [70% (v/v) ethanol for 1 min, 30% (v/v) sodium hypochlorite for 5 min, and washing three times in distilled water] were arranged on a filter paper in Petri dishes (diameter: 9 cm, 10 seeds/Petri dish). The filter papers were moistened with either 5 mL of distilled water (control) or 5 mL of PPP suspensions. Next, N-PPP, P-PPP, Mix-PPP, and Raw-PPP were applied in the following three different doses: 0.05% (m/v), 0.5% (m/v), and 5% (m/v). Each Petri dish was closed but not sealed, and then the seeds were left to germinate (25 °C in the dark). After 4 days, each seed with visible roots was counted as germinated, and the length of the primary root was measured. Relative seed germination and relative root length were determined in comparison with the control and finally used to calculate the index of germination (IG%) as follows: IG% = [(% seed germination) × (% root length)]/100.

### 2.4 Pot experiments

To evaluate the agricultural feasibility of PPP supplementation and determine its effect on the early vegetative growth of oilseed rape, we conducted an outdoor pot experiment from June 24 to July 26 in 2021 at the Institute of Biology, University of Szeged. The average temperature during the growth period was 26.08 °C, and the total precipitation was 48 mm (detailed weather conditions are presented in Fig. S1). A 600-g mixture of the required PPP and potting soil [Mr. Garden, AGRO CS Slovakia a.s., Lučenec, Slovakia; pH = 5.5, N: 0.1% (m/m), $P_2O_5$: 0.01% (m/m), $K_2O$: 0.03% (m/m), organic matter: 75% (m/m)] was prepared and added into a plastic pot (volume: 2 L, height: 13.1 cm, width: 16 cm, depth: 16 cm). Next, N-PPP, P-PPP, Mix-PPP, and Raw-PPP were applied in three different doses, viz., 0.05% (m/m), 0.5% (m/m), and 5% (m/m). The control experiment did not contain any of the PPPs. The bottom of the pot was covered with a piece of filter paper to prevent soil loss. All seeds were surface-sterilized [(70% (v/v) ethanol and 30% (v/v) sodium hypochlorite] before pregermination at 26 °C for 48 h (Feigl et al., 2019; Meng et al., 2021). Then, seven *Brassica* seedlings were transferred to the soil surface of each prefilled pot. All pots were randomly placed in an outdoor bright area protected from direct sunlight and were regularly reorganized (Fig. S2). The initial moisture content was 70% (m/m), and plants were watered each day if necessary. Excess rainwater was collected and returned to the pots (Görlach et al., 2021). Each experiment was conducted in triplicate; hence, 39 pots were cultivated. Plants were harvested after 32 days.

### 2.5 Plant morphology measurements

Plant habit (cm) was determined on the 32nd day as the maximum height that a *Brassica* plant can maintain against gravity, indicated by the distance between the soil surface in the pot and the highest point of the plant. Fresh and dry weights (g) of the plant material were measured using a digital scale immediately after harvesting on the 32nd day and after drying for 3 days at 70 °C, respectively. The number of *Brassica* leaves was counted manually and expressed as pieces/plant (Borbély et al., 2021; Feigl et al., 2016). Leaf size ($cm^2$) and total leaf area ($cm^2$/plant) were determined using a grid and ImageJ software (National Institute of Mental Health, Bethesda, Maryland, USA) (Feigl et al., 2016).

### 2.6 Western blot detection of tyrosine-nitrated proteins

After the completion of the 32-day cultivation period, protein extracts were prepared from *Brassica* root tissues, protein separation was performed on 12% acrylamide gels (sodium dodecyl sulfate-polyacrylamide gel electrophoresis, SDS-PAGE), and then Western blotting was conducted according to procedures described by Feigl et al. (2015).

### 2.7 Statistical analysis

Data were statistically analyzed using the SigmaPlot 11.0 software (Systat Software Inc., Erkrath, Germany) by one-way analysis of variance (ANOVA; $P < 0.05$) and Duncan's multiple range test, and results were expressed as mean values with standard errors.

## 3 Results and discussion

### 3.1 In vitro phytotoxicity of PPPs

In vitro experimental setups were used to evaluate the phytotoxicological effects of raw and nutrient-fortified PPPs on the germination and early root development of *Brassica* seedlings. Compared with the control, Raw-PPP supplementation significantly increased the IG% value (109.6%) at 0.05% concentration, whereas higher concentrations (0.5% and 5%) gradually reduced the IG% values (63.9% and 8.9%, respectively), indicating a concentration-dependent inhibitory effect (Fig. 1). The decreased IG% of oilseed rape observed at higher PPP concentrations can be explained by the fact that phenolic compounds, primarily concentrated in the peel of pomegranate, can be phytotoxic (especially lipophilic phenolics) and cause plant necrosis at higher concentrations (Campos et al., 2022). The effect of nutrient-fortified PPP supplementation was less detrimental than that of Raw-PPP supplementation. Application of N-PPP and P-PPP significantly increased the IG% value at both 0.05% and 0.5% concentrations. Mix-PPP supplementation at 0.05% and 0.5% concentrations exerted a positive effect (121.3%) and no effect (103.5%) on IG%, respectively.

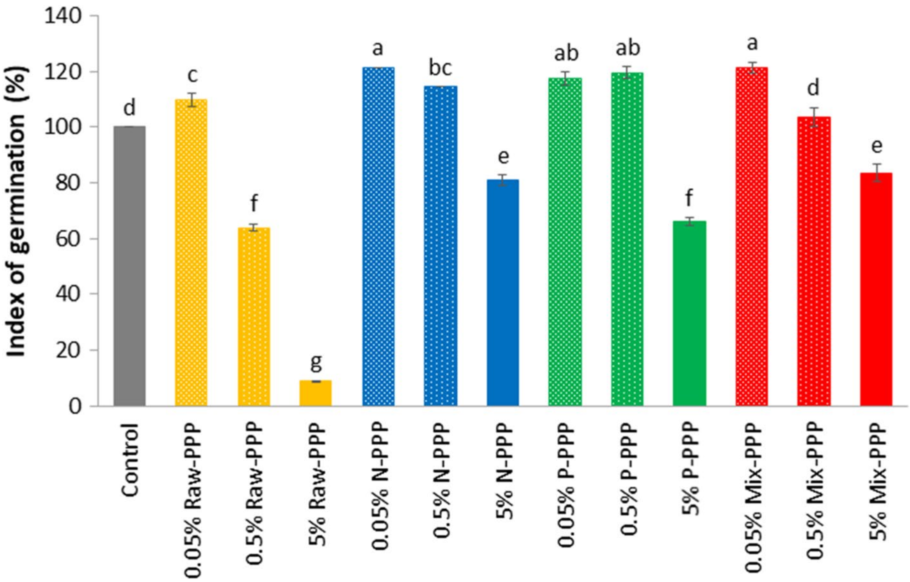


**Fig. 1** In vitro germination index of *Brassica napus* in the presence of raw and nutrient-fortified pomegranate peel powders (PPPs) at different concentrations (0.05%, 0.5%, and 5%). Different letters in each column represent significant differences according to Duncan's multiple range test ($P < 0.05$)

The strong inhibitory effect observed at high concentrations of Raw-PPP was alleviated after supplementation with N-PPP, P-PPP, and Mix-PPP probably due to either the longer production process of nutrient-fortified PPPs (including several preparatory steps), which could decrease the concentration of phenolics, or the presence of adsorbed macronutrients, which could be desorbed during germination and become available for the seedlings. The uptake of P by plants from soil and fertilizer sources is often hindered by the low bioavailability of P from such complex minerals (e.g., hydroxylapatites, struvite, or vivianite) it forms with $Fe^{3+}$, $Al^{3+}$, $Fe^{2+}$, or $Ca^{2+}$, and thus, long-lasting and easily accessible P sources are more preferable for plant production (Stavkova & Maroušek, 2021). In this regard, P-PPP and Mix-PPP could be considered as agrochemically favorable alternatives to mineral fertilizers, since P is stored in an organic matrix, and once in the soil, P would presumably be released slowly as the organic matter decomposes.

Because the supplementation of PPPs did not affect the relative seed germination of oilseed rape (all germination rates were almost 100% in any of the examined PPP concentrations), all the observed alterations in IG% values can be exclusively attributed to the significant changes in the primary root length of the seedlings (Table S1), wherein low concentrations of PPPs slightly promoted root elongation, whereas high concentrations resulted in reduced primary root lengths.

### 3.2 Effect of PPPs on plant morphology

An outdoor pot experiment was conducted to evaluate the agricultural feasibility of applying PPP as a biofertilizer.

Although plant habit remained largely unaffected by the addition of PPPs, some treatments (i.e., 0.05% Mix-PPP and 5% Mix-PPP) tended to slightly promote plant height (Fig. 2). Furthermore, the addition of 5% Raw-PPP and 0.5% Mix-PPP resulted in taller *Brassica* plants than those in the control soil.

We also conducted a detailed morphological analysis on the 32-day-old plants to disclose the nature of PPP effect on the early vegetative development of oilseed rape.

PPP supplementation in the soil affected the growth of the two main organs of rapeseed plants in different manners (Table 1). Although N-PPP supplementation did not exert any significant effect on the fresh weight of shoots compared with the control (5.55 g), supplementation with Raw-PPP, P-PPP, and Mix-PPP could slightly enhance shoot biomass. The most significant changes were observed with the addition of 5% Raw-PPP and 0.5% Mix-PPP, with the shoot biomass being 8.60 g and 8.49 g, respectively. Conversely, PPP supplementation exerted no or rather negative effects on root biomass according to the statistical analysis. Root fresh weight values most similar to those in the control (0.52 g) were measured only in samples treated with 5% N-PPP, 0.05% P-PPP, and 0.5% P-PPP. Remarkably, the most defined negative effects on the fresh weight of roots were detected in pots supplemented with Mix-PPP (0.18–0.32 g). Similar to the in vitro phytotoxicity results (Table S1), root development was obstructed by the presence of PPPs in soil but to a significantly lesser extent, suggesting that the soil system modulates their negative effects. In fact, a recent study described about the ameliorative properties of soils in freshly plastic-contaminated environments (Mészáros et al., 2022).

The pattern of changes in the dry weights of shoots and roots was analogous to those observed in the case of fresh weight (Table 1), which is consistent with the fact that the water content of the total plant was not significantly affected by PPP application. Despite the comparable tendencies to fresh shoot weights, PPP application did not cause any

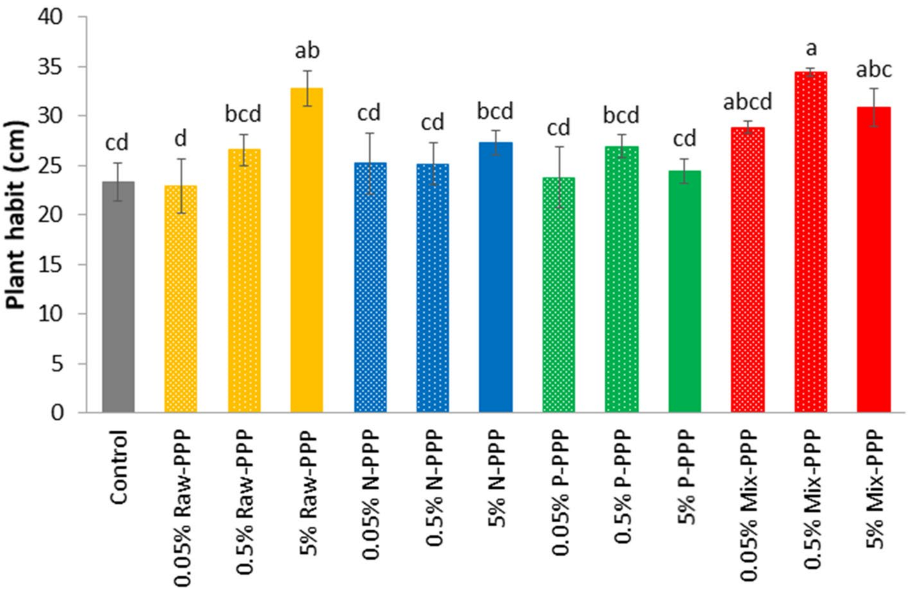


**Fig. 2** Plant habit of 32-day-old *Brassica napus* grown in soils supplemented with raw and nutrient-fortified pomegranate peel powders (PPPs) at different concentrations (0.05%, 0.5%, and 5%). Different letters in each column represent significant differences according to Duncan's multiple range test ($P < 0.05$)

**Table 1** Shoot and root fresh weights, shoot and root dry weights, shoot/root ratios, and water content of 32-day-old *Brassica napus* grown in soils supplemented with raw and nutrient-fortified pomegranate peel powders (PPPs) at different concentrations (0.05%, 0.5%, and 5%)

| Treatment | Fresh weight | | | Dry weight | | | Water content of the total plant (m/m%) |
|---|---|---|---|---|---|---|---|
| | Shoot (g) | Root (g) | Shoot/root ratio (%) | Shoot (g) | Root (g) | Shoot/root ratio (%) | |
| Control | 5.55 ± 0.73b | 0.52 ± 0.17ab | 10.67 | 0.48 ± 0.07n.s | 0.08 ± 0.02a | 6.00 | 90.77 ± 0.77n.s |
| 0.05% Raw-PPP | 6.54 ± 0.52ab | 0.26 ± 0.03 cd | 25.15 | 0.49 ± 0.05n.s | 0.03 ± 0.01cd | 16.33 | 92.35 ± 1.33n.s |
| 0.5% Raw-PPP | 7.02 ± 0.8ab | 0.34 ± 0.06abcd | 20.65 | 0.57 ± 0.07n.s | 0.04 ± 0.01bcd | 14.25 | 91.71 ± 1.40n.s |
| 5% Raw-PPP | 8.60 ± 0.54a | 0.37 ± 0.04abcd | 23.24 | 0.69 ± 0.05n.s | 0.04 ± 0.01bcd | 17.25 | 91.86 ± 0.73n.s |
| 0.05% N-PPP | 5.31 ± 0.48b | 0.40 ± 0.08abc | 13.28 | 0.41 ± 0.05n.s | 0.08 ± 0.01a | 5.13 | 91.42 ± 1.04n.s |
| 0.5% N-PPP | 5.83 ± 0.45b | 0.37 ± 0.05abcd | 15.76 | 0.52 ± 0.05n.s | 0.06 ± 0.01abcd | 8.67 | 90.65 ± 1.42n.s |
| 5% N-PPP | 6.23 ± 0.48b | 0.50 ± 0.06ab | 12.46 | 0.56 ± 0.05n.s | 0.08 ± 0.01a | 7.00 | 90.49 ± 1.14n.s |
| 0.05% P-PPP | 6.59 ± 0.31ab | 0.47 ± 0.05abc | 14.02 | 0.55 ± 0.04n.s | 0.07 ± 0.01ab | 7.86 | 91.22 ± 0.99n.s |
| 0.5% P-PPP | 6.62 ± 0.45ab | 0.55 ± 0.06a | 12.04 | 0.63 ± 0.06n.s | 0.09 ± 0.01a | 7.00 | 89.96 ± 1.39n.s |
| 5% P-PPP | 6.33 ± 0.89b | 0.36 ± 0.07abcd | 17.58 | 0.61 ± 0.09n.s | 0.06 ± 0.01abc | 10.17 | 89.99 ± 1.06n.s |
| 0.05% Mix-PPP | 6.91 ± 0.73ab | 0.18 ± 0.03d | 38.39 | 0.44 ± 0.06n.s | 0.03 ± 0.00d | 14.67 | 93.37 ± 0.78n.s |
| 0.5% Mix-PPP | 8.49 ± 0.85a | 0.30 ± 0.05bcd | 28.30 | 0.66 ± 0.08n.s | 0.04 ± 0.01cd | 16.50 | 92.04 ± 1.60n.s |
| 5% Mix-PPP | 7.38 ± 0.79ab | 0.32 ± 0.04bcd | 23.06 | 0.54 ± 0.07n.s | 0.03 ± 0.01cd | 18.00 | 92.60 ± 1.04n.s |

Different letters in each column represent significant differences according to Duncan's multiple range test ($P < 0.05$)

*n.s.* no significant difference

statistically significant changes in dry shoot weights. As observed in the case of fresh root weights, dry root weights were mostly reduced in response to PPP applications. The maximum decrease was measured in soils supplemented with Raw-PPP (0.03–0.04 g) and Mix-PPP (0.03–0.04 g), whereas dry root weights under supplementation with N-PPP and P-PPP were primarily similar to that in the control (0.08 g).

Compared with the control, both fresh and dry shoot/root ratios confirmed that all the concentrations of PPPs promoted the growth of aboveground plant parts over the underground parts (Table 1), with the most remarkable increases observed in Mix-PPP-supplemented soils.

Further analysis of the effects of PPP application on the aboveground plant parts showed that in general compared with the control, leaf counts were slightly increased with the addition of PPPs (Table 2), where 0.5% Mix-PPP supplementation led to the development of a significantly higher number of leaves.

In general, leaf sizes (Table 2) were slightly reduced by supplementation with N-PPP and P-PPP, whereas none or weak positive responses were induced by supplementation with Mix-PPP (excluding 0.05% Mix-PPP) and Raw-PPP (the highest leaf size at 5% Raw-PPP). Interestingly, the individual changes within the same PPP treatment group appeared to be dependent on the dosage with Raw-PPP and N-PPP additions, larger leaves were detected with increasing PPP concentrations, but with P-PPP and Mix-PPP additions, the largest leaves were observed at medium PPP concentrations. These different tendencies (although not always supported by ANOVA) might be explained by the differences in the production processes of N-PPP and P-PPP: To improve its phosphate adsorption and retention ability, P-PPP was activated with iron chloride ($FeCl_3$) during production (Bellahsen et al., 2021a). Although iron is an essential micronutrient for plant development, excess iron can cause toxicity and disrupt cell homeostasis, resulting in tissue damage (Santana et al., 2014). Hu et al. (2017) investigated the phytotoxicity of ferric ions ($Fe^{3+}$) in the concentration range of 20–100 mg/L and reported the maximum chlorophyll content in *Citrus*

**Table 2** Leaf count, leaf size, and total leaf area of 32-day-old *Brassica napus* grown in soils supplemented with raw and nutrient-fortified pomegranate peel powders (PPPs) at different concentrations (0.05%, 0.5%, and 5%)

| Treatment | Number of leaves (pcs) | Leaf size (cm$^2$) | Total leaf area (cm$^2$) |
|---|---|---|---|
| Control | 4.8 ± 0.21bc | 26.49 ± 2.49abc | 111.16 ± 12.65c |
| 0.05% Raw-PPP | 4.9 ± 0.18bc | 25.37 ± 1.85abc | 121.50 ± 9.14bc |
| 0.5% Raw-PPP | 5.1 ± 0.23abc | 25.63 ± 2.15abc | 121.62 ± 13.05bc |
| 5% Raw-PPP | 5.0 ± 0.16abc | 31.58 ± 2.34a | 157.79 ± 8.33a |
| 0.05% N-PPP | 5.5 ± 0.18ab | 19.84 ± 1.72c | 108.59 ± 11.28c |
| 0.5% N-PPP | 5.5 ± 0.23ab | 21.62 ± 1.83bc | 106.87 ± 9.19c |
| 5% N-PPP | 5.2 ± 0.16abc | 23.95 ± 1.83bc | 116.44 ± 9.22bc |
| 0.05% P-PPP | 5.5 ± 0.17ab | 21.49 ± 1.40bc | 111.68 ± 5.82c |
| 0.5% P-PPP | 4.6 ± 0.24c | 25.12 ± 2.02abc | 104.75 ± 8.24c |
| 5% P-PPP | 5.2 ± 0.17abc | 21.43 ± 2.07bc | 96.85 ± 12.70c |
| 0.05% Mix-PPP | 5.1 ± 0.24abc | 21.00 ± 1.55c | 105.28 ± 10.38c |
| 0.5% Mix-PPP | 5.7 ± 0.21a | 27.49 ± 2.18ab | 150.74 ± 17.77ab |
| 5% Mix-PPP | 5.1 ± 0.22abc | 25.98 ± 2.09abc | 122.59 ± 14.45bc |

Different letters in each column represent significant differences according to Duncan's multiple range test ($P < 0.05$)

*maxima* leaves at a medium $Fe^{3+}$ concentration. Our results are consistent with these observations, suggesting that exceeding an optimal dosage of P-PPP, as a potential source of ferric ions, also retards the leaf development of oilseed rape.

In most cases, the total leaf area values (Table 2) remained at or near the control level (111.16 $cm^2$/plant). Only 5% Raw-PPP (157.79 $cm^2$/plant) and 0.5% Mix-PPP (150.74 $cm^2$/plant) treatments significantly enhanced the total leaf area per plant due to their increased leaf size and leaf number, respectively.

Interestingly, the plant morphology results indicated that 5% Raw-PPP demonstrated one of the best growth-promoting performances on aboveground plant parts. However, this could also be achieved using Mix-PPP at 0.5% concentration, which represents an order-of-magnitude lower dosage and can make both the production and application of PPPs easier. Consequently, Mix-PPP can be used as a more economically justified biofertilizer.

There is extremely scant literature on the effect of PPP supplementation on plant growth and morphology. Dayarathna and Karunarathna (2021) reported that plant height, leaf area, and dry biomass of okra (*A. esculentus* L.) were significantly enhanced by Raw-PPP supplementation (applied as 1:1 = PPP:soil for 8 weeks). According to Mercy et al. (2014), increasing doses of Raw-PPP promoted the growth of fenugreek (*T. foenum-graceum* L.) plants cultivated in soil-filled pots for 45 days. In another soil experiment, Raw-PPP-treated (dosages ranging from 0.02 to 0.06%) sage herb (*S. officinalis* L.) showed increased fresh and dry biomass after 270 days (Abd-Rabbu et al., 2021). Another study showed that the application of Raw-PPP-coated urea fertilizer (doses ranging from 30 to 100%) increased the biomass and grain yield of maize (*Z. mays* L.) after 65 days (Sabahi et al., 2017). Obviously, the experimental design, plant species, growth period, applied PPP dosage, and used method are quite variable in those studies, making it difficult to compare the results with our observations. However, our work suggests that different plant species respond differently to the presence of PPPs, and hence future studies on PPP application should include more plant species.

### 3.3 Effect of PPPs on the pattern of protein tyrosine nitration in the root

The results of Western blotting revealed protein tyrosine nitration, which is a nitro-oxidative stress-induced post-translational modification, in *Brassica* root tissues after 32 days of cultivation. Depending on the level of reactive oxygen and nitrogen species, tyrosine-nitrated proteins are formed through various physiological and pathological processes, and therefore, provide valuable information on the stress state of plants (Feigl et al., 2020).

Figure 3 shows the results of Western blot analysis. Compared with control plants, the nitration of a protein band with higher weight (approximately 50 kDa) decreased in all samples from plants grown in PPP-supplemented pots, possibly due to protein degradation processes (Molnár et al., 2020). PPP supplementation exerted a treatment-dependent mixed effect in the nitration of proteins with a mass of approximately 40 kDa, whereas the overall protein tyrosine nitration increased in the lower molecular weight zone (approximately 25 kDa and below) in response to every treatment. In general, the highest nitration signals were detected at 0.5% PPP concentrations. As this increase could already be observed in plants grown in Raw-PPP-supplemented pots, it could be attributed to the presence of PPP itself and not to nutrient fortification. However, P-PPP- and Mix-PPP-treated samples also exhibited slightly higher nitration levels than other samples, suggesting that P-PPP induces stronger nitro-oxidative responses than N-PPP.

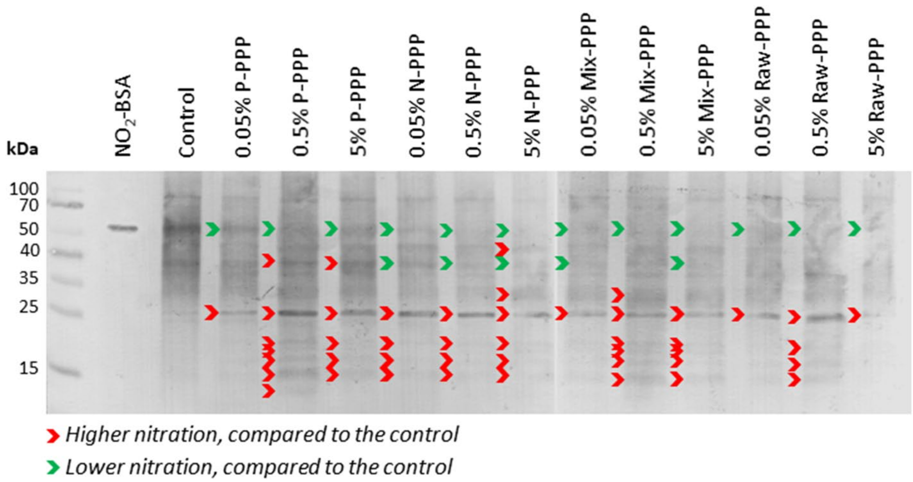


**Fig. 3** Immunoblot demonstrating protein tyrosine nitration in the roots of 32-day-old *Brassica napus* grown in soils supplemented with raw and nutrient-fortified pomegranate peel powders (PPPs) at different concentrations (0.05%, 0.5%, and 5%). Commercial nitrated bovine serum albumin ($NO_2$-BSA) was used as the positive control, and the molecular marker is shown as a protein weight (kDa) indicator. Red arrows show protein bands with increased nitration compared with the control, and green arrows show protein bands with decreased nitration compared with the control

In plants subjected to heavy metal stress, the level of protein tyrosine nitration is often increased, indicating a state of stress (Gzyl et al., 2016; Feigl et al., 2015, 2016, 2019, 2020; Kolbert et al., 2020). However, the simultaneous appearance and disappearance of immunopositive bands result in a changed pattern of protein tyrosine nitration and can demonstrate a tolerance mechanism to heavy metal exposure (Feigl et al., 2019, 2020). In the present study, there were distinct changes in the protein tyrosine nitration pattern in all treated samples compared with the control (Fig. 3). Considering the generally positive growth responses (Fig. 2, Tables 1, 2), this pattern rearrangement suggested that the plants were acclimated to the presence of PPPs in the soil, although the treatment also induced nitro-oxidative responses in the roots (most pronounced when supplemented at 0.5% concentration). On the basis of these observations, it can be assumed that although PPP supplementation alters the nitrated protein homeostasis, it did not induce serious stress responses in the roots of the tested plants.

## 4 Conclusions

Available nitrogen and phosphorus are essential for the proper development of plants and, hence, for the production of nutritionally valuable food. In addition to the feasibility of nutrient-fortified PPPs as biofertilizers, their preparation process not only decreases the amount of pomegranate food waste, but also reduces the (potentially risky) high nitrogen and phosphorus content of dairy wastewaters, contributing to a more sustainable agricultural management in regard to environmental and health safety. We confirmed that the unfavorable in vitro effects of Raw-PPP might be avoided using nutrient-fortified PPPs. Furthermore, several doses of PPP supplementation were beneficial for the model crop plant when applied in the soil, promoting the development of aboveground plant parts rather than underground parts. PPPs also proved to induce no or low-level stress in oilseed rape. Although their production must be scaled up in a cost-effective manner (a significant challenge from an engineering point of view), and the appropriate dosage also requires further evaluation, including more plant species and soil types, our study implies that nutrient-fortified PPPs produced from plant residues and industrial wastewater could be potential candidates for sustainable soil fertility improvement in the future agricultural technology.

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**Data availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Declaration**

**Conflict of interest** The authors declare no conflict of interest.



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