Sonchus oleraceus residue improves nutritive and health-promoting value of common bean (Phaseolus vulgaris L.) : a metabolic study

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Sonchus oleraceus residue improves nutritive and health-promoting value #of common bean (Phaseolus vulgaris# #L.): A metabolic study

Mahmoud O. Hassan, Ahmed M. Saleh, and Hamada AbdElgawad

J. Agric. Food Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.7b05821 • Publication Date (Web): 17 Feb 2018

Just Accepted

ACS Publications

Journal of Agricultural and Food Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Title page

Sonchus oleraceus residue improves nutritive and health-promoting value of common bean (*Phaseolus vulgaris* L.): A metabolic study

Mahmoud O. Hassan[1,#], Ahmed M. Saleh[2,3,#,*], Hamada AbdElgawad[1,4]

[1]Department of Botany and Microbiology, Faculty of Science, Beni Suef University, 62521 Beni-Suef, Egypt

[2] Biology Department, Faculty of Science Yanbu, Taibah University, King Khalid Rd., Al Amoedi, 46423 Yanbu El-Bahr, Saudi Arabia

[3] Department of Botany and Microbiology, Faculty of Science, Cairo University, Giza 12613, Egypt

[4] Laboratory for Molecular Plant Physiology and Biotechnology, Department of Biology, University of Antwerp, Groenenborgerlaan 171, B-2020, Antwerp 2020, Belgium

# These authors contributed equally to this manuscript and share first authorship

[*]Corresponding Authors asaleh@sci.cu.edu.eg

Running title: *S. oleraceus* residue promotes health benefits of *P. vulgaris*

Keywords:

Sonchus oleraceus, Phaseolus vulgaris, Organic farming, Phenolic compounds, Fatty acids, Amino acids, Vitamens, Antioxidant capacity

Abstract

This study was conducted to evaluate the use of the phenolic-rich *Sonchus oleraceus* residue as an environmentally safe approach to induce the nutritive and health-promoting values of common bean (*Phaseolus vulgaris* L. cv. Bronco). *S. oleraceus* shoot residue, at rates of 150 and 300 g m^{-2}, has improved soil fertility via accumulation of soil macronutrients, organic matter, organic carbon and total phenolics. The growth and yield of bean was significantly increased. Moreover, chemical composition of the treated seeds was significantly altered, whereas higher levels of total antioxidant capacity, proteins, carbohydrates and most of the individual phenolic acids, flavonoids, vitamins, essential amino acids, and unsaturated fatty acids were recorded. Interestingly, concentration dependent effect was also observed, for instance a lower saturated-to-unsaturated fatty acid ratio was only observed in case of the lower residue rate. These findings recommend the use of *S. oleraceus*, in organic farming of bean to enhance the health benefits of the produced beans.

1. Introduction

Common beans (*Phaseolus vulgaris* L.) are widely consumed as a dietary source for proteins, minerals, carbohydrate, vitamins, fibers, antioxidants and nutraceuticals [1,2]. Like other legumes, beans are believed to play a beneficial role in prevention of obesity, diabetes, cardiovascular diseases and cancer [3,4]. These pharmaceutical benefits are mainly linked to the polyunsaturated fatty acids, vitamins, phenolic and flavonoid ingredients of the seed [5–8]. Actually, the normal level of these compounds in plant sources, including beans, is less than the optimal for health maintenance [9], thus improving the accumulation of such health-beneficial phytochemicals in beans is worthwhile. Moreover, maximizing the productivity of crop plants is a demand to supply the growing population with sufficient food. Therefore, more efforts should be devoted to improve quantity and quality of crops, while bearing in mind the environmental safety.

In the last decades, organic farming has been attracted much attention as an alternative approach to minimize the environmental hazards of synthetic fertilizers and pesticides in the agro-ecosystems [10]. Regarding the public health, organic fruits and vegetables are believed to be health-promoting, where it contains lower level of nitrate and pesticide residues and higher contents of vitamins, secondary metabolites and essential amino acids when compared with conventional ones [11,12]. Unfortunately, due to restriction in the use of synthetic pesticides and fertilizers, plant diseases and nutrients management are major threats for organic agriculture [13]. In this regard, there are evidence that exogenous application of plant phenolic compounds could play a beneficial role in controlling phytopathogens, either directly by altering the ultrastructure and physiology of the pathogen or indirectly by inducing the innate plant defence mechanisms [14,15]. Moreover, the release of phenolic compounds into soil has been found to be beneficial for plants suffering nutrient deficiency by helping in solubilization and release of nutrient, like P and Fe, into the soil solution [16]. These

compounds could also improve the nitrogen use efficiency through inhibition of biological nitrification and therefore minimizing the loss of nitrogen from the soil [17]. From a metabolic point of view, exogenous application of phenolic compounds has been reported to promote the accumulation of carbohydrates, proteins, antioxidants, phenolic acids, flavonoids in the receptor plant [18–20]. Therefore, utilization of phenolic-rich plant residues in organic farming could not only help in plant diseases and nutrients management, but also could improve the nutritive and health-promoting value of crop plants.

Among plants characterized by high level of phenolic compounds in their tissues is the annual sowthistle (*Sonchus oleraceus* L.), one of the cosmopolitan weed species dominating the weed communities in the agro-ecosystems and orchards [21–23]. During the hoeing practices in the agro-ecosystem, the Egyptian farmers uproot this plant and leave it onto or mix it directly with the soil during the tillage process. This process may affect the incoming crop, in a species-dependent manner, due to the potential release of phytochemicals from the residues. In this regard, larger-seeded species were reported to be highly tolerant to the effects of plant residues [24], the trait that could qualify beans to withstand and give vigorous growth in the presence of plant residue, particularly at low or moderate doses. Recently, we have reported that inclusion of *S. oleraceus* residue into the soil has enhanced its organic matter and nutrients [25]. Moreover, the impact of *S. oleraceus* residue or its extract on growth and some metabolic factors of certain crop and weed species has been investigated, under greenhouse conditions [25,26]. Nevertheless, no field data is available about the effect of *S. oleraceus* residue on the nutritive and health-promoting value of crop plants. Accordingly, in the present study we aim, to present a field investigation about the implication of *S. oleraceus* residue on the yield quantity and quality of common beans (*Phaseolus vulgaris* L.), with a special emphasis on the accumulation of health-beneficial phytochemicals. To achieve this purpose, the regulatory role of the manure of this weed on productivity and accumulation of individual minerals, primary and

secondary metabolites and non-enzymatic antioxidants in seeds of *P. vulgaris* was assessed, under field conditions.

2. Materials and Methods

2.1. Residue collection and site description

Fresh shoots of *S. oleraceus* were collected during the growing season (October 2014 to April 2015), from different localities in the agro-ecosystems of Beni Suef governorate, Egypt. Collection process was performed during the early fruiting stage in order to facilitate the distinction of *S. oleraceous* from its congener *S. asper*. Furthermore, at this stage, plants mostly produce the maximum amounts of bioactive metabolites [27]. Plant materials were put in polyethylene bags and immediately carried to the laboratory for further processing. Once in the lab, fruits and inflorescences were completely removed and the plant materials were air dried and stored in refrigerator at 4 °C until use. The study site was about 12 km south west to Beni Suef University (29° 09.13 N, 31° 08.36 E). The field soil was classified as sandy clay loam. Soil physicochemical characteristics were pH (1:2.5 H_2O) 7.97, EC (mS cm^{-1}) 0.40, organic matter 2.97%, organic carbon 1.69 (%), available N, P, K and Zn 99, 2.83, 607.5, 5.98 mg kg^{-1}, respectively, and the field capacity was about 39%.

2.2. Field experiment

First, the field of study was mechanically homogenised and subdivided into quadrates/plots (2×2 m^2, each). Under field conditions, *S. oleraceus* residues were amended with the soil at the rates 150 and 300 g m^{-2}, whereas the un-amended quadrates were left as control. These amounts were mostly coinciding with low and moderate amounts of the residue (Hassan et al., 2014). Thirty-two common bean seeds (*Phaseolus vulgaris* L. cv. Bronco) were then sown within each m^2 (at 2.5 cm in depth), buried and watered. The emerging seedlings were thinned to the most similar sixteen ones. The effects of *S. oleraceus* residue were evaluated in the bean field from 1st October 2015 to the end of January 2016. This period may synchronize with the time of cultivation of this crop. During the

period of cultivation, weeding process was performed twice in order to avoid the interference of weeds with the cultivated crop. The overall experiment was conducted in a complete randomized design (CRD), including two different levels of manure, with four replicates. At harvest, the most similar and dominant pods from each quadrate were carefully removed from the vegetative parts. Some growth parameters, particularly shoot length, biomass and leaf area, were estimated. Besides, yield parameters including the number, length, fresh weights of pods per individual and per plot were measured. Some other individuals were left until the seed output to record the number of seeds formed per pod and the seed yield per plot. For biochemical analysis, seeds were collected from the parent plant at the senesces stage to obtain the mature dry seeds. The seeds were washed, cleaned, finely ground and stored at -20°C till processing.

2.2.1. Soil analysis

At the harvest process, three soil samples were collected from each quadrate at 0–30 cm depth to form a one composite sample for each treatment. Soil properties including pH, electrical conductivity (EC), organic carbon (OC), organic matter (OM), available nutrients such as nitrogen, phosphorus, potassium, and Zinc were determined. These parameters were determined using the standard methods [28].

2.3. Analyses of metabolites

2.3.1. Determination of individual Minerals

A 0.1 g dry plant sample was digested in a HNO_3/H_2O solution (5:1 v/v) in an oven according to Agusa et al. [29]. The macro-minerals and trace elements were determined (ICP-MS, Finnigan Element XR, Scientific, Bremen, Germany). Standards were also prepared in 1% nitric acid.

2.3.2. Quantitative estimation of Sugars

Mono, di and poly saccharides in the legumes were measured by using high-performance liquid chromatography (HPLC) following Hamad et al. [30]. 150 mg of dry plant tissue was vigorously

homogenized in 2 ml of acetonitrile:water solution (1:1 v/v) for 5 min. Samples were boiled for sure denaturation of sugar enzymes. After 15 min of incubation at 55–60 °C in water bath, filtration was performed through Whattman NO. 1 filter paper. Further, 20 ml of solvents were also added to the remaining pulp. The column temperature was adjusted at 30 °C and injection volume was 20 µl. Filtered mobile phase (mixture of acetonitrile and HPLC-grade water (75:25 v/v) at 1 ml min^{-1}). The existing sugars were identified and quantified on the basis of peak areas and comparison with a calibration curve obtained with the corresponding standards ranging from 1 to 10 mg 100 ml^{-1} of the same extracting solution. Extraction and determination of soluble, insoluble and total carbohydrates were performed following the method described by Clark and Switzer [31].

2.3.3. Organic acids analysis

The quantitative determination of organic acids was performed according to [30]. Breifly, 500 mg of powdered seeds were homogenized in 0.1% phosphoric acid containing 0.003% butylated hydroxyanisole. After centrifugation at 14,000 rpm and 4 °C for 30 min, the supernatants were passed through Millipore micro filters. Organic acids were detected by HPLC using a SUPELCOGEL C-610H column (300 mm × 7.8 mm, Supelco, Sigma, St. Louis, MO, USA) coupled to UV detection system set at 210 nm (LaChrom L-7455 diode array, LaChrom, Tokyo, Japan). Phosphoric acid (0.1%) at a flow rate of 0.45 ml min^{-1} was used as a mobile phase. The concentrations of individual organic acids were calculated using a calibration curve of corresponding standards.

2.3.4. Amino acids quantification

A 0.2 g dry plant samples were homogenized in a 5 ml 80% aqueous ethanol using a MagNALyser (Roche, Vilvoorde, Belgium) for 1 min, at 5000 rpm [32]. After centrifugation at 14,000 rpm for 20 min, the supernatant was evaporated under vacuum, and the pellet was re-suspended in 5 ml chloroform. Immediately, the residue was re-extracted with 1ml deionized water (HPLC grade) and

the centrifugation was repeated. The supernatant was re-mixed with the pellet suspended in chloroform and then centrifuged for 10 min. The aqueous phase was filtered using Millipore micro filters (0.2 μM pore size). The amino acids were determined by using a Waters Acquity UPLC-tqd system (Milford, Worcester County MA, USA) equipped with a Sinha BEH amide 2.1 × 50 column.

2.3.5. Analysis of fatty acid

Fatty acids quantification was performed by gas chromatography according to [33]. A 200 mg dry plant samples were used to obtain lipophilic fractions via extraction in aqueous methanol (1:1 w/v) at 25 °C until discoloration of the tissues occurred. In this process, nonadecanoic acid was used as an internal standard. GC/MS analysis was carried out on a Hewlett Packard 6890, MSD 5975 mass spectrometer (Hewlett Packard, Palo Alto, CA, USA), with an HP-5 MS column (30 m × 0.25 mm × 0.25 mm). Lipids were identified with the NIST 05 database and Golm Metabolome Database, http://gmd.mpimp-golm.mpg.de).

2.3.6. Phenolics and flavonoids determination

A 0.1 g plant sample was extracted in 250 ml acetone-water solution (4:1 v/v) at room temperature for 24 on orbital shaker. The extracts were filtered, centrifuged and supernatant was concentrated using a rotary evaporator (IKA-WERKE-RV06ML, Stanfer, Germany) to obtain DPF hydroxyacetone crude extract. The residue was dissolved in HPLC grade methanol to give a concentration of 1000 ppm and measured by injecting 20 μl sample into Shimadzu HPLC system (SCL-10 A vp, Shimadzu Corporation, Kyoto, Japan) [30]. The HPLC system consisted of a diode-array detector and a Lichrosorb Si-60, 7μm, 3 x 150 mm column. The mobile phase consisted of water-formic acid (90:10, v/v); and acetonitrile/water/formic acid (85:10:5, v/v/v). individual phenolic compounds and flavonoids were quantified by comparing with the corresponding standards.

For determination of total phenols and flavonoids, 100 mg dry plant samples were homogenized in 0.5 ml 80% aqueous ethanol. After centrifugation, pellet was washed twice each with 0.5 ml 80% ethanol and supernatants were pooled. Total phenolic content was determined using Folin–Ciocalteu assay according to Saleh et al. [18]. Gallic acid was used as a standard. The total flavonoid content was estimated using the modified aluminum chloride calorimetric method [30] with quercetin as a standard.

2.3.7. Quantitative analysis of Ascorbate and tocopherols

Ascorbate content was determined in 100 mg dry plant tissue by reversed phase HPLC. Separation and detection processes by UV detector were performed following Potters et al. [34] protocol. Total ascorbate concentration was determined after reduction with DTT (0.04 M) for 10 min at room temperature. The redox status was calculated as the ratio of the reduced form to the total concentration. Tocopherols were extracted in hexane [35]. The extract was dried under vacuum conditions (CentriVap concentrator, Labconco, KS, USA) and was re-suspended in hexane. Tocopherols were separated and quantified by HPLC (Shimadzu, Hertogenbosch, Netherlands) using normal phase conditions (Particil Pac 5 µm column material, length 250 mm, i.d. 4.6 mm). 5,7-dimethyltocol (DMT; 5 ppm) was used as an internal standard. Data were analyzed with Shimadzu Class VP 6.14 software provided by the HPLC system (Shimadzu, Tokyo, Japan).

2.4. Determination of total Antioxidant Capacity (TAC)

TAC were measured in 100 mg samples as mentioned by Benzie and Strain [36]. Known volume of sample was mixed with reaction mixture in a micro-plate. The mixture was kept on ice for 20 min and read at 600nm. Antioxidant Trolox was used as standard.

2.5. Statistical analyses

Data collected were analysed using the SPSS Statistics software package, version 20 (IBM Corporation, USA). The Kolmogorov-Smirnov and Levene tests for homogeneity of variances were

178 applied to check the data for normality and homoscedasticity. The data that presented normality and
179 homoscedasticity were analyzed by the parametric statistic, using one-way ANOVA followed by
180 Tukey's test for multiple comparisons of means. The data that were not normal or heteroscedastic
181 were analysed via Kruskal-Wallis H test. Values were compared at 0.05 probability level. For data of
182 metabolite analyses, cluster analysis was performed by using Pearson distance metric by using
183 MultiExperiment Viewer (MeV)™ 4 software package (version 4.5, Dana-Farber Cancer Institute,
184 Boston, MA, USA).

185 **3. Results and Discussion**

186 ***3.1. S. oleraceus residue improves soil fertility***

187 To reveal the effect of *S. oleraceus* residue on the physicochemical properties of the soil; pH, EC,
188 OC, OM, total phenolics and some mineral nutrients were analyzed at harvest (Table 1). No
189 significant effect of *S. oleraceus* amendment on soil pH was observed, however EC, OC and OM
190 were significantly increased at the two rates of residue amendments (150 and 300 g m^{-2}). In this
191 regard, we have reported that *S. oleraceus* shoot residue had resulted in a rate-dependent increase in
192 the EC, OC and OM of the soil under greenhouse conditions [25]. Similar improvements of soil OC and
193 OM was reported in response to residue amendments of different crop plants [37–39].

194 A manifold increase in the content of phenolic compounds was observed in soil amended with *S.*
195 *oleraceus*, with the highest concentration of phenolics recorded at the higher residue rate (Table 1).
196 This result could be attributed to the high phenolic content, 560 µg g^{-1}dry weight, of *S. oleraceus*
197 shoot residues (Table 2). Among these, ferulic acid and catechol were the most abundant, while
198 sinapic acid and resorcinol were detected in moderate amounts. In the same context, previous studies
199 of our and others have been reported the presence of various phenolic acids and flavonoids in *S.*
200 *oleraceus* [23,40]. However, some kind of variability is existed in phenolic profile of *S. oleraceus*, which

may be attributed to the genetic variability and conditions of the agroecosystem. In a related research, we have detected that phenolic compounds released from *S. oleraceus* residues show some persistence in soil, where the concentration of individual phenolic compounds peaks at 8 d after residue application then decreased gradually [25]. Moreover, Martens [39] reported significant improvements in the concentration of total phenolic acids in Webster soil treated with residues of several plant species. He recorded that the content of phenolic acids decreased gradually over 84 d of residue incorporation.

Regarding the level of available soil macronutrient, both rates of *S. oleraceus* residue had improved the concentration of exchangeable N and P, however no significant difference was observed between the two rates. In contrast, the level of available soil K did not significantly affected by incorporation of *S. oleraceus* residue. The increase in available soil macronutrient could be attributed to mineralization of the residue and/or improvement of minerals desorption from soil particles. Supporting this idea, the study of Iqbal [41] revealed a positive impact of crop residues in reducing the strong absorption of P by soil particles. Moreover, several investigations had recorded elevated levels of exchangeable N, P and K in soil incorporated with residues of different plans, as compared with residue free soil [37,38,42]. In addition, some reports point to a role of plant residues in promoting the processes of biological nitrogen fixation and nodulation [38,43,44].

3.2. S. oleraceus residue improves growth and yield of P. vulgaris

Incorporation of *S. oleraceus* residue at the rates of 150 and 300 g m^{-2} soil had no evident impact on shoot growth parameters except for the leaf area that is significantly improved (Table 3). Such improved leaf area could enhance yield production through its impact on the process of seed filling [45]. Supporting this idea, both lower and higher rates of residue significantly improved the fruit yield of common bean by about 65 and 33%, respectively, as compared with the control (Table 3). However,

seed yield was significantly enhanced by the lower residue rate only. Such enhancement in yield production may be a logical consequence of the enhanced soil fertility due to release of nutrients during residue decomposition. Beside, residue incorporation in the soil is believed to improve water retention, nodulation and biological nitrogen fixation [43,44]. In accordance with our results, Bahrani et al. [38] reported significant increases in yield and seed weight of red bean (*P. vulgaris* L.) cultivated in soil amended with wheat residue. Moreover, similar improvement in yield of wheat and tomato was obtained in soil treated with different plant residues [37,42]. From another point of view, the higher productivity of plants grown in soil amended with *S. oleraceus* residue may be attributed to the phenolics ingredient of the decomposed residue that could reduce the susceptibility of plants to phytopathogens, therefore maintain yield, either directly by harming soil born phytopathogens or indirectly through induction of plant defense mechanisms. Supporting this idea, Al-Wakeel et al.[15] reported that priming of sunflower seeds with two phenolic compounds, salicylic acid and coumarin, has induced the plant systemic resistance and reduced the incidence of charcoal rot disease. In addition, phenolic rich extracts from several plant species have been reported to possess strong antifungal activities [14].

Our results manifested that shoot biomass and the number of seeds produced in both treated and control plants remained statistically constant. However, the total yield with respect to fruit yield and seed weight was stimulated to some extent in the residue-amended soils. This result suggests that the treated plants seem to succeed in their reproductive allocation. This could be attributed to the enhanced availability of soil organic matter and nutrients, which reduces competition among plants for such resources. Supporting this interpretation competitive interactions is known to affect reproductive allocation [46].

3.3. S. oleraceus soil amendment modulates the chemical composition of P. vulgaris seeds

3.3.1. Accumulation of minerals in bean seeds is enhanced by S. oleraceus residue

Quantitative analysis of minerals in bean seeds showed that K was the most abundant one followed by P and Mg (Table 3). Incorporation of *S. oleraceus* residue had significantly improved the accumulation of all the measured minerals, except for P that had inhibited by the higher residue rate (300 g m^{-2} soil). Seeds produced in soil amended with 150 g m^{-2} *S. oleraceus* residue had accumulated about 11, 13 and 36 % K, Mg and P, respectively, over those produced in soil with no residue. In this context, different varieties of *P. vulgaris* show a high variability in their mineral composition [47,48]. The elevated levels of seed minerals as affected by *S. oleraceus* residue could be attributed to higher availability and/or improved uptake of these minerals via plant roots. In this regard, plant residues have been reported to increase the level of exchangeable minerals in the soil solution [37,38,42].

3.3.2. S. oleraceus residue improves the contents of soluble and insoluble sugars while decreases the levels of the major organic acids

Data presented in Table 4 show that soluble sugars detected in bean seeds followed the order fructose > glucose > sucrose. Incorporation of *S. oleraceus* residue in the soil had no significant effect on the levels of glucose and fructose, while significantly improved the accumulation of sucrose and total soluble sugars at both rates of soil amendments. Moreover, both lower and higher rates of *S. oleraceus* residues had significantly increased the content of insoluble sugars by about 23 and 31%, respectively, as compared with the control. The induced accumulation of soluble and insoluble sugars in seeds produced in *S. oleraceus* amended soil may be attributed to a higher rate of photosynthesis due to improved soil N and P. Supporting this interpretation, the relationship between N and P nutrition and the photosynthetic efficiency of plants has been extensively discussed in the literature [49,50]. Moreover, phenolic ingredient of the residue may play a role in promoting the photosynthetic

machinery and accumulation of sugars. In this regard, some investigations reported that lower concentrations of exogenously applied phenolics might exert positive effects on accumulation of photosynthetic pigments and Rubisco activity [18,51].

Six organic acids were detected in bean seeds including citric, fumaric, isobutyric, malic, oxalic and succinic acids (Table 4). Among these, malic acid was the predominant compound where it accounted for about 70% of the organic acid pool in seeds. The levels of citric, oxalic and succinic acids did not significantly affected by incorporation of *S. oleraceus* residue. However, seeds produced in *S. oleraceus* amended soils contained significantly lower levels of malic and isobutyric acids and higher amounts of fumaric acid, relative to those produced in residue free soil. In consistence with these results, malic acid was recorded as the major organic acid in seeds of different varieties of *P. vulgaris* [52]. Beside its role as flavour enhancers, organic acids, except for oxalic, possess health benefits for human such as restraining the undesired microflora and helping in absorption of nonheme Fe from vegetarian food [53].

3.3.3. Individual amino acids is differentially influenced by S. oleraceus residue

Regarding the essential amino acid, leucine was the most dominant while phenylalanine, lysine and histidine were detected in moderate amounts (Table 4). On the other hand, glutamate was the abundant non-essential amino acid. These results are in agreement with Baptista et al. [8] who reported that leucine, phenylalanine and lysine were the major essential amino acids, whereas glutamic and aspartic acids were the predominant non-essential amino acids detected in *P. vulgaris* and two other pulse species.

Total protein content of *P. vulgaris* seeds was improved as affected by *S. oleraceus* residues (Table 4). Such improvement was accompanied with significant enhancements in the total content of essential amino acids, at both residue rates. Seeds produced in soil amended with the lower residue

rate (150 g m^{-2}) contained significantly higher levels of lysine and phenylalanine, while the higher values of leucine was observed in case of the higher rate of soil amendment. On the other hand, the level of total non-essential amino acids was only improved by the higher residue rate (300 g m^{-2}). Such improvement was a consequence for the elevated levels of glutamate, glutamine and alanine. The elevated levels of some amino acids in seeds produced in soil amended with *S. oleraceus* residue suggest a positive impact for residue on the biosynthesis of amino acids probably through enhancements in available soil N (Table 1), nitrogen fixation and/or nodulation. In this context, previous investigations have revealed that decaying plant residues could enrich the exchangeable soil N and promote the processes of biological nitrogen fixation and nodulation [25,38,43,44]. Moreover, The improved accumulation of essential amino acids, as affected by *S. oleraceus* residue could enhance the nutritive value of bean, being one of the major dietary source of plant-based proteins [4].

3.3.4. S. oleraceus residue induces the levels of MUFA and PUFA but not SFA

To reveal the impact of *S. oleraceus* amendment on the fatty acids (FA) composition of bean seeds, individual saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids were analyzed by GC/MS. Results presented in Table 4 show that palmitic acid (C16:0) was the most dominant SFA, while α-linolenic (C18:3, cis-$\Delta^{9,12,15}$) and linoleic acids (C18:2, cis-$\Delta^{9,12}$) were the abundant PUFA. These results are in accordance with previous studies on bean seeds [8,54,55]. Dissimilar to the results of Baptista et al. [8], palmitoleic acid (C16:1, cis-Δ^{7}) was the most dominant MUFA that detected in the current study.

The lower rate of *S. oleraceus* residue (150 g m^{-2}) has decreased the level of total SFA by about 12%, however the higher amendment rate exerted a positive impact on the accumulation of SFA, about 22% over the control. Moreover, about 16 and 11 % improvements in the content of total MUFA and PUFA, respectively, were observed by the lower rate of residue. On contrary, the accumulation of

316 UFA was negatively influenced by the higher residue rate (Table 4). Interestingly, a lower
317 SFA/(MUFA + PUFA) ratio was obtained in case of the lower *S. oleraceus* residue rate, as compared
318 to the control. This result suggests a greater health-promoting value for seeds obtained after
319 incorporation of *S. oleraceus* residue in the soil (150 g m^{-2}), where lower SFA/(MUFA + PUFA) ratio
320 in diet is correlated with cardioprotective effects [56]. The alteration in the accumulation of fatty acids
321 as affected by *S. oleraceus* treatment may suggest a possible impact on the process of dark
322 respiration, whereas malonyl-CoA, the precursor of fatty acids biosynthesis, is produced from ATP-
323 dependent carboxylation of the glycolytic pathway intermediate, acetyl-CoA[57].

324 *3.3.5. Accumulation of phenolics and flavonoids is upregulated by S. oleraceus residue*

325 Data presented in Table 5 show the phenolic and flavonoid composition of bean seeds. Eight phenolic
326 acids, were detected including caffeic, chlorogenic, ferulic, gallic, p-coumaric, protocatechuic,
327 resorcinol and syringic acids. This result is more or less similar to previous studies on bean varieties
328 [7,58,59]. Among the detected phenolic acids, gallic acid was the most abundant, while p-coumaric and
329 ferulic were found in moderate amounts. In this regard, gallic, vanillic, and chlorogenic acids were
330 the predominant phenolic acids identified in two varieties of *P. vulgaris* L., pinto and black beans [59].
331 Moreover, in a comparative study conducted using fifteen variety of dry bean (*P. vulgaris* L.), Luthria
332 and Pastor-Corrales [58] reported that ferulic acid was the most dominant phenolic acid followed by p-
333 coumaric and sinapic acids. On the other hand, the present results revealed the presence of five
334 flavonoids in bean seeds, among them catechin was the predominant one followed by quercetin and
335 its derivative, rutin. These results are quite similar to those in the literature, however distinct
336 differences were recorded among the different varieties of beans [59,60].

337 Incorporation of *S. oleraceus* residue in the soil significantly improved the concentration of the
338 majority of individual phenolic acids and flavonoids as well as their totals in the produced seeds

(Table 5). The lower rate of residue amendment (150 g m^{-2}) has improved the total phenolic and flavonoid contents by 23 and 13%, respectively, while the higher rate (300 g m^{-2}) has resulted in about 43 and 15% increase in their contents. The elevated levels of phenolic compounds in response to *S. oleraceus* residue could be attributed to the phenolic ingredient of the residue, where exogenously applied phenolics have been reported to upregulate the phenylpropanoids pathway leading to accumulation of phenolic acids and flavonoids [18–20]. Similarly, in a pot experiment, Gomaa et al. [26] reported that application of *S. oleraceus* shoot residue at the rate of 10 g kg^{-1} soil has improved the accumulation of individual and total flavonoids in shoot of *Trifolium alexandrinum*. From health prospective, the elevated levels of phenolic acids and flavonoids, as affected by *S. oleraceus* soil amendment, could improve the health-promoting capacity of bean, where phenolic compounds extracted from different bean varieties have been reported to have antioxidant, anticancer and antimutaginic activities [5,6,61]. Moreover, phenolic compounds have been found to inhibit the digestion and intestinal absorption of carbohydrates and fats by altering glucosidase and lipase activities, therefore could provide anti-diabetic properties [62,63].

3.3.6. Induced Vitamins and TAC as affected by S. oleraceus residue

Vitamins are among the most beneficial ingredient of fruits and vegetable [64]. In the present study, the levels of ascorbic acid (vitamin C) and the different forms of tocopherols (vitamin E) were measured in bean seeds (Table 5). The results revealed that ascorbic acid was the most dominant vitamin followed by α-tocopherol. The lower rate of *S. oleraceus* amendment (150 g m^{-2}) had significantly improved the accumulation of ascorbic acid in bean seeds, while the higher rate of residue was inhibitory. Regarding the levels of the detected tocopherols, both rates of *S. oleraceus* residue have a significant positive impact on the levels of individual, except for γ-tocopherol, and total tocopherols. In accordance with the present results, similar pattern of vitamins were detected in different varieties

of *P. vulgaris*, however a little difference in the concentration and relative dominance of these vitamins was documented [8,55]. The enhanced accumulation of vitamins C and E in response to *S. oleraceus* soil amendment could contribute to the health-promoting properties of the produced seeds through improvement of its antioxidant capacity [65]. Supporting this explanation, seeds produced in soil amended with both rates of *S. oleraceus* residue showed higher TAC as compared with those produced in soil with no residue (Table 5). Beside, such improved TAC could be attributed to the higher phenolic and flavonoid contents, where these compounds are known for their free radical scavenging activity [66]. In this regard, Amarowicz et al. [61] reported a significant correlation between total phenolic compounds and TAC of seed extracts from different types of bean. Moreover, a strong positive correlation was existed between the antioxidant capacity of different plant species and their contents of both phenolics and flavonoids [14].

3.4. The effect S. oleraceus residue on metabolite accumulation is concentration-dependent and metabolite-specific

A cluster analysis was performed to explore more details about the metabolic implications provoked by *S. oleraceus* soil amendment on chemical composition of bean seeds. (Figure 1). Measured metabolites can be separated into five clusters based on their response to different residue rates. The first cluster, mainly organic acids, were decreased by lower or higher residue rate. Total primary metabolites, total flavonoid, P, ascorbic acid and unsaturated fatty acids, were strongly increased by the low residue rate but decreased by the higher one (the second cluster). The third cluster showed increased minerals and sucrose levels by the high residue rate; however, the fourth cluster, mainly other sugars, were increased by both residue rates and the last cluster contained mainly non-essential amino acids which were strongly increased by the higher residue rate but decreased by the lower one. These results clearly indicate that the effect of *S. oleraceus* residue on accumulation of

phytochemicals in bean seeds was a function of the concentration applied and the type of metabolites. Similarly, previous studies reported that the effect of pure phenolic compounds or plant residues on metabolism of the receptor plant is dependent on the concentration applied [18,40,67].

4. Conclusion

The findings of the present study suggest that incorporation of phenolic-rich plant residues, such as *S. oleraceus*, in the soil is a promising ecofriendly approach in organic farming of *P. vulgaris*. Such soil amendment could support soil fertility by increasing the level of exchangeable soil macronutrients such as N and P that may be released through mineralization of the residue, improvement of minerals desorption from soil particles and/or the effect of residue's bioactive phytochemicals on the processes of biological nitrogen fixation and nodulation. Moreover, *S. oleraceus* soil amendment, in a concentration-dependent manner, could improve the health-beneficial value of the produced seeds as indicated by the elevated levels of certain minerals, essential amino acids, USFA, vitamins, phenolic acids and flavonoids. The improved accumulation of these health-beneficial compounds could be attributed to phenolic compounds of the residue, which are reported for their regulatory effect on the biosynthesis of primary and secondary plant metabolites.

References

(1) Siddiq, M.; Ravi, R.; Harte, J. B.; Dolan, K. D. Physical and functional characteristics of selected dry bean (*Phaseolus vulgaris* L.) flours. *LWT-Food Sci. Technol.* **2010**, *43* (2), 232–237.

(2) Stagnari, F.; Maggio, A.; Galieni, A.; Pisante, M. Multiple benefits of legumes for agriculture sustainability: an overview. *Chem. Biol. Technol. Agric.* **2017**, *4* (1), 2.

(3) Chung, H.-J.; Liu, Q.; Hoover, R.; Warkentin, T. D.; Vandenberg, B. In vitro starch digestibility, expected glycemic index, and thermal and pasting properties of flours from pea, lentil and chickpea cultivars. *Food Chem.* **2008**, *111* (2), 316–321.

(4) Hayat, I.; Ahmad, A.; Masud, T.; Ahmed, A.; Bashir, S. Nutritional and Health Perspectives

410 of Beans (*Phaseolus vulgaris* L.): An Overview. *Crit. Rev. Food Sci. Nutr.* **2014**, *54* (5), 580–592.

411 (5) de Mejía, E. G.; Castaño-Tostado, E.; Loarca-Piña, G. Antimutagenic effects of natural
412 phenolic compounds in beans. *Mutat. Res. Toxicol. Environ. Mutagen.* **1999**, *441* (1), 1–9.

413 (6) Madhujith, T.; Amarowicz, R.; Shahidi, F. Phenolic antioxidants in beans and their effects on
414 inhibition of radical-induced DNA damage. *J. Am. Oil Chem. Soc.* **2004**, *81* (7), 691–696.

415 (7) Siah, S. D.; Konczak, I.; Agboola, S.; Wood, J. A.; Blanchard, C. L. In vitro investigations of
416 the potential health benefits of Australian-grown faba beans (*Vicia faba* L.): chemopreventative
417 capacity and inhibitory effects on the angiotensin-converting enzyme, α-glucosidase and lipase. *Br. J.*
418 *Nutr.* **2012**, *108* (S1), S123–S134.

419 (8) Baptista, A.; Pinho, O.; Pinto, E.; Casal, S.; Mota, C.; Ferreira, I. M. P. L. V. O.
420 Characterization of protein and fat composition of seeds from common beans (*Phaseolus vulgaris* L.),
421 cowpea (*Vigna unguiculata* L. Walp) and bambara groundnuts (*Vigna subterranea* L. Verdc) from
422 Mozambique. *J. Food Meas. Charact.* **2017**, *11* (2), 442–450.

423 (9) Brandt, K.; Mølgaard, J. P. Organic agriculture: Does it enhance or reduce the nutritional
424 value of plant foods? *J. Sci. Food Agric.* **2001**, *81* (9), 924–931.

425 (10) Peigné, J.; Casagrande, M.; Payet, V.; David, C.; Sans, F. X.; Blanco-Moreno, J. M.; Cooper,
426 J.; Gascoyne, K.; Antichi, D.; Bàrberi, P.; et al. How organic farmers practice conservation
427 agriculture in Europe. *Renew. Agric. Food Syst.* **2015**, *31* (February), 1–14.

428 (11) Bourn, D.; Prescott, J. A Comparison of the Nutritional Value , Sensory Qualities , and Food
429 Safety of Organically and Conventionally Produced Foods. *Crit. Rev. Food Sci. Nutr.* **2002**, *42* (1),
430 1–34.

431 (12) del Amor, F. M.; Serrano-Martinez, A.; Fortea, I.; Núñez-Delicado, E. Differential effect of
432 organic cultivation on the levels of phenolics, peroxidase and capsidiol in sweet peppers. *J. Sci. Food*
433 *Agric.* **2008**, *88* (5), 770–777.

434 (13) Finckh, M. R.; Schulte-Geldermann, E.; Bruns, C. Challenges to organic potato farming:
435 disease and nutrient management.. *Potato Res.* **2006**, *49* (1), 27–42.

436 (14) Mohamed, M. S. M.; Saleh, A. M.; Abdel-Farid, I. B.; El-Naggar, S. A. Growth, hydrolases
437 and ultrastructure of *Fusarium oxysporum* as affected by phenolic rich extracts from several
438 xerophytic plants. *Pestic. Biochem. Physiol.* **2017**, *141*, 57–64.

439 (15) Al-Wakeel, S. M.; Gabr, M. M. A.; Abu-El-Soud, W. M.; Saleh, A. M. Coumarin and
440 salicylic acid activate resistance to *Macrophomina phaseolina* in *Helianthus annuus*. *Acta Agron.*
441 *Hungarica* **2013**, *61* (1), 23–35.

442 (16) Masaoka, Y.; Kojima, M.; Sugihara, S.; Yoshihara, T.; Koshino, M.; Ichihara, A. Dissolution
443 of ferric phosphate by alfalfa (*Medicago sativa* L.) root exudates. *Plant Soil* **1993**, *155* (1), 75–78.

444 (17) Subbarao, G. V; Nakahara, K.; Ishikawa, T.; Ono, H.; Yoshida, M.; Yoshihashi, T.; Zhu, Y.;
445 Zakir, H.; Deshpande, S. P.; Hash, C. T.; et al. Biological nitrification inhibition (BNI) activity in
446 sorghum and its characterization. *Plant Soil* **2013**, *366* (1–2), 243–259.

(18) Saleh, A. M.; Madany, M. M. Y.; González, L. The effect of coumarin application on early growth and some physiological parameters in faba bean (*Vicia faba* L .). *J. Plant Growth Regul.* **2015**, *34* (2), 233–241.

(19) Saleh, A. M.; Madany, M. M. Y. Coumarin pretreatment alleviates salinity stress in wheat seedlings. *Plant Physiol. Biochem.* **2015**, *88*, 27–35.

(20) Abu El-Soud, W.; Hegab, M. M.; AbdElgawad, H.; Zinta, G.; Asard, H. Ability of ellagic acid to alleviate osmotic stress on chickpea seedlings. *Plant Physiol. Biochem.* **2013**, *71*, 173–183.

(21) Hutchinson, I. A. N.; Colost, J.; Lewin, R. A. The biology of canadian weeds.: 63. *Sonchus asper* (L.) Hill and *S. oleraceus* L. *Can. J. plant Sci.* **1984**, *64* (3), 731–744.

(22) Abd El-Gawaad, A. M. Ecology and allelopathic control of Brassica tournefortii in reclaimed areas of the Nile Delta, Egypt. *Turk. J. Botany* **2014**, *38* (2), 347–357.

(23) Al Juhaimi, F.; Ghafoor, K.; Ahmed, I. A. M.; Babiker, E. E.; Özcan, M. M. Comparative study of mineral and oxidative status of *Sonchus oleraceus*, *Moringa oleifera* and *Moringa peregrina* leaves. *J. Food Meas. Charact.* **2017**, *11* (4), 1745–1751.

(24) Xiong, S.; Nilsson, C. *The effects of plant litter on vegetation: a meta-analysis*; Wiley Online Library, 1999; Vol. 87.

(25) Hassan, M. O.; Gomaa, N. H.; Fahmy, G. M.; González, L.; Hammouda, O.; Atteya, A. M. Influence of Sonchus oleraceus L. Residue on Soil Properties and Growth of Some Plants. *Philipp. Agric. Sci.* **2014**, *97* (4), 368–376.

(26) Gomaa, N. H.; Hassan, M. O.; Fahmy, G. M.; Gonzalez, L.; Hammouda, O.; Atteya, A. M. Flavonoid profiling and nodulation of some legumes in response to the allelopathic stress of Sonchus oleraceus L. *Acta Bot. Brasilica* **2015**, *29* (4), 553–560.

(27) Çirak, C.; Radušienė, J.; Janulis, V.; Ivanauskas, L. Secondary metabolites in *Hypericum perfoliatum*: variation among plant parts and phenological stages. *Bot. Helv.* **2007**, *117* (1), 29–36.

(28) Jones, J. *Laboratory guide for conducting soil tests and plant analysis*; CRC press, 2001.

(29) Agusa, T.; Kunito, T.; Yasunaga, G.; Iwata, H.; Subramanian, A.; Ismail, A.; Tanabe, S. Concentrations of trace elements in marine fish and its risk assessment in Malaysia. *Mar. Pollut. Bull.* **2005**, *51* (8), 896–911.

(30) Hamad, I.; Abdelgawad, H.; Al Jaouni, S.; Zinta, G.; Asard, H.; Hassan, S.; Hegab, M.; Hagagy, N.; Selim, S. Metabolic analysis of various date palm fruit (*Phoenix dactylifera* L.) cultivars from Saudi Arabia to assess their nutritional quality. *Molecules* **2015**, *20* (8), 13620–13641.

(31) Clark, J. M.; Switzer, R. L. *Experimental Biochemistry*, 2nd editio.; W.H. Freeman & Company, San Francisco, 1977.

(32) Sinha, A. K.; Giblen, T.; AbdElgawad, H.; De Rop, M.; Asard, H.; Blust, R.; De Boeck, G. Regulation of amino acid metabolism as a defensive strategy in the brain of three freshwater teleosts in response to high environmental ammonia exposure. *Aquat. Toxicol.* **2013**, *130*, 86–96.

(33) Torras-Claveria, L.; Berkov, S.; Codina, C.; Viladomat, F.; Bastida, J. Metabolomic analysis of bioactive Amaryllidaceae alkaloids of ornamental varieties of Narcissus by GC--MS combined with k-means cluster analysis. *Ind. Crops Prod.* **2014**, *56*, 211–222.

(34) Potters, G.; Horemans, N.; Bellone, S.; Caubergs, R. J.; Trost, P.; Guisez, Y.; Asard, H. Dehydroascorbate influences the plant cell cycle through a glutathione-independent reduction mechanism. *Plant Physiol.* **2004**, *134* (4), 1479–1487.

(35) Siebert, K. J. Modeling the flavor thresholds of organic acids in beer as a function of their molecular properties. *Food Qual. Prefer.* **1999**, *10* (2), 129–137.

(36) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* **1996**, *239* (1), 70–76.

(37) Ghadikolayi, A. K.; Kazemeini, S. A.; Bahrani, M. J. Wheat yield and soil properties as influenced by crops residues and nitrogen rates. *Aust. J. Crop Sci.* **2015**, *9* (9), 853–858.

(38) Bahrani, M. J.; Salehi, F.; Kazemeini, S. A. Yield and yield components of two irrigated red bean cultivars and some soil properties as influenced by wheat residues and nitrogen management. *Arch. Agron. Soil Sci.* **2012**, *58* (2), 179–187.

(39) Martens, D. A. Plant residue biochemistry regulates soil carbon cycling and carbon sequestration. *Soil Biol. Biochem.* **2000**, *32* (3), 361–369.

(40) Gomaa, N. H.; Hassan, M. O.; Fahmy, G. M.; González, L.; Hammouda, O.; Atteya, A. M. Allelopathic effects of *Sonchus oleraceus* L. on the germination and seedling growth of crop and weed species. *Acta Bot. Brasilica* **2014**, *28* (3), 408–416.

(41) Iqbal, S. M. Effect of crop residue qualities on decomposition rates, soil phosphorus dynamics and plant phosphorus uptake [PhD thesis]., University of Adelaide, 2009.

(42) Galieni, A.; Stagnari, F.; Speca, S.; D'Egidio, S.; Pagnani, G.; Pisante, M. Management of crop residues to improve quality traits of tomato (*Solanum lycopersicum* L.) fruits. *Ital. J. Agron.* **2017**, *12* (1), 59–62.

(43) Mandal, S. M.; Chakraborty, D.; Dey, S.; Mandal, S. M.; Chakraborty, D.; Dey, S. Phenolic acids act as signaling molecules in plant- microbe symbioses Phenolic acids act as signaling molecules in plant-microbe symbioses. *Plant Signal. Behav. ISSN* **2010**, *5* (4), 359–368.

(44) Kumar, K.; Goh, K. M. Crop residues and management practices: effects on soil quality, soil nitrogen dynamics, crop yield, and nitrogen recovery. *Adv. Agron.* **1999**, *68*, 197–319.

(45) Bewley, J. D.; Bradford, K. J.; Hilhorst, H. W. M.; Nonogaki, H. *Seeds: Physiology of development, germination and dormancy, 3rd edition*; 2013; Vol. 9781461446.

(46) Bazzaz, F. A.; Ackerly, D. D.; Reekie, E. G.; others. *Reproductive allocation in plants*; CABI Publishing Wallingford, 2000; Vol. 2.

(47) Shimelis, E. A.; Rakshit, S. K. Proximate composition and physico-chemical properties of improved dry bean (*Phaseolus vulgaris* L.) varieties grown in Ethiopia. *LWT - Food Sci. Technol.* **2005**, *38* (4), 331–338.

(48) Nwokolo, E. Common bean (*Phaseolus vulgaris* L.). In *Food and Feed from Legumes and Oilseeds*; Nwokolo, E., Smartt, J., Eds.; Springer US: Boston, MA, 1996; pp 159–172.

(49) Fredeen, A. L.; Raab, T. K.; Rao, I. M.; Terry, N. Effects of phosphorus nutrition on photosynthesis in Glycine max (L.) Merr. *Planta* **1990**, *181* (3), 399–405.

(50) Evans, J. R. Photosynthesis and nitrogen relationships in leaves of C 3 plants. *Oecologia* **1989**, *78* (1), 9–19.

(51) Khodary, S. E. A. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt-stressed maize plants. *Int. J. Agric. Biol* **2004**, *6* (1), 5–8.

(52) John, K. M. M.; Luthria, D. Amino Acid, Organic Acid, and Sugar Profiles of 3 Dry Bean (*Phaseolus vulgaris* L.) Varieties. *J. Food Sci.* **2015**, *80* (12), C2662–C2669.

(53) Nawirska-Olszanska, A.; Biesiada, A.; Soktowska, A.; Kucharska, A. Z. Characteristics of organic acids in the fruit of different pumpkin species. *Food Chem.* **2014**, *148*, 415–419.

(54) Lo Turco, V.; Potortì, A. G.; Rando, R.; Ravenda, P.; Dugo, G.; Di Bella, G. Functional properties and fatty acids profile of different beans varieties. *Nat. Prod. Res.* **2016**, *30* (19), 2243–2248.

(55) Yoshida, H.; Tomiyama, Y.; Mizushina, Y. Characterization in the fatty acid distributions of triacylglycerols and phospholipids in kidney beans (*Phaseolus vulgaris* L.). *Journal of Food Lipids*. 2005, pp 169–180.

(56) Livingstone, K. M.; Lovegrove, J. A.; Givens, D. I. The impact of substituting SFA in dairy products with MUFA or PUFA on CVD risk: evidence from human intervention studies. *Nutr. Res. Rev.* **2012**, *25* (2), 193–206.

(57) Brown, A.; Slabas, A.; Rafferty, J. Fatty Acid Biosynthesis in Plants — Metabolic Pathways, Structure and Organization. *Lipids Photosynth. SE - 2* **2010**, *30*, 11–34.

(58) Luthria, D. L.; Pastor-Corrales, M. A. Phenolic acids content of fifteen dry edible bean (*Phaseolus vulgaris* L.) varieties. *J. food Compos. Anal.* **2006**, *19* (2), 205–211.

(59) Xu, B.; Chang, S. K. C. Total phenolic, phenolic acid, anthocyanin, flavan-3-ol, and flavonol profiles and antioxidant properties of pinto and black beans (*Phaseolus vulgaris* L.) as affected by thermal processing. *J. Agric. Food Chem.* **2009**, *57* (11), 4754–4764.

(60) Lin, L.-Z.; Harnly, J. M.; Pastor-Corrales, M. S.; Luthria, D. L. The polyphenolic profiles of common bean (*Phaseolus vulgaris* L.). *Food Chem.* **2008**, *107* (1), 399–410.

(61) Amarowicz, R.; TROSZYŃSKA, A.; BARYŁKO-PIKIELNA, N.; Shahidi, F. Polyphenolics extracts from legume seeds: correlations between total antioxidant activity, total phenolics content, tannins content and astringency. *J. Food Lipids* **2004**, *11* (4), 278–286.

(62) Ikarashi, N.; Takeda, R.; Ito, K.; Ochiai, W.; Sugiyama, K. The inhibition of lipase and glucosidase activities by acacia polyphenol. *Evidence-Based Complement. Altern. Med.* **2011**, *2011*.

(63) Sharma, I.; Aaradhya, M.; Kodikonda, M.; Naik, P. R. Antihyperglycemic, antihyperlipidemic

556 and antioxidant activity of phenolic rich extract of *Brassica oleraceae* var gongylodes on
557 streptozotocin induced Wistar rats. *Springerplus* **2015**, *4* (1), 212.

558 (64) Brandt, K.; Leifert, C.; Sanderson, R.; Seal, C. J. Agroecosystem management and nutritional
559 quality of plant foods: The case of organic fruits and vegetables. *CRC. Crit. Rev. Plant Sci.* **2011**, *30*
560 (1–2), 177–197.

561 (65) Traber, M. G.; Stevens, J. F. Vitamins C and E: beneficial effects from a mechanistic
562 perspective. *Free Radic. Biol. Med.* **2011**, *51* (5), 1000–1013.

563 (66) Duthie, G.; Crozier, A. Plant-derived phenolic antioxidants. *Curr. Opin. Lipidol.* **2000**, *11* (1),
564 43–47.

565 (67) Madany, M. M. Y.; Saleh, A. M. Phytotoxicity of Euphorbia helioscopia L. on *Triticum*
566 *aestivum* L. and *Pisum sativum* L.. *Ann. Agric. Sci.* **2015**, *60* (1), 141–151.

567

Figure Caption

Figure 1. Heatmap of metabolite accumulation in common bean seeds produced in either control or S. oleraceus amended soils at the rates of 150 and 300 g m-2. The relative accumulation patterns are shown in the heatmap based on the average value (n=4) for each metabolite. Red and blue colors indicate lower and higher concentrations, respectively.

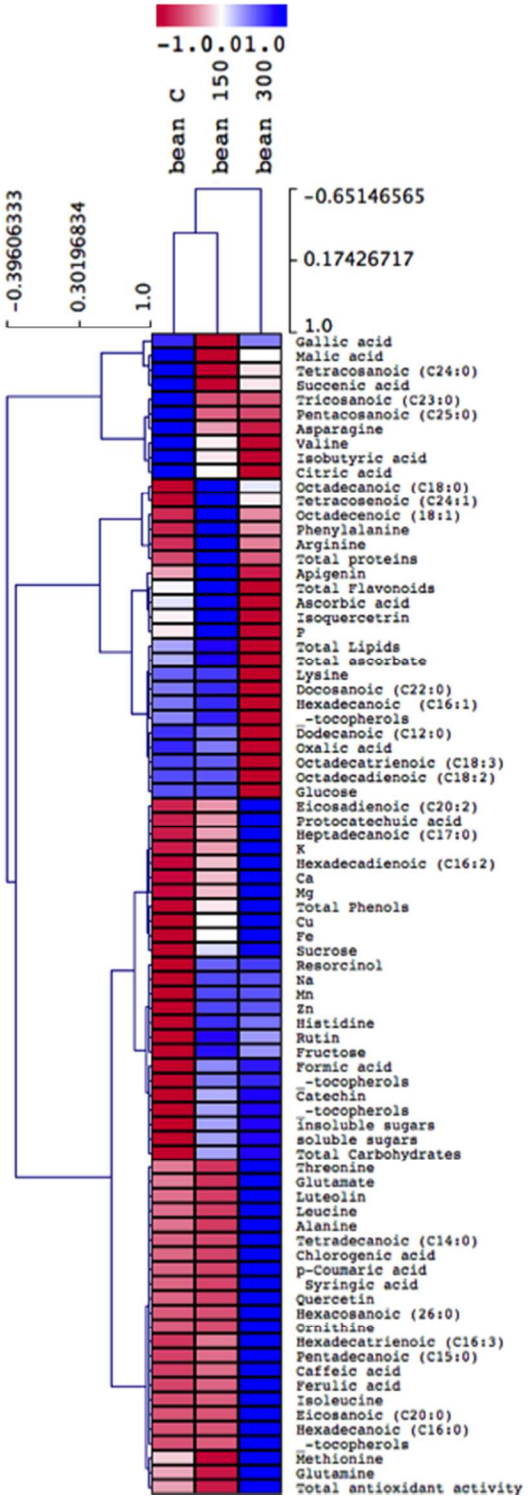


Table 1. Influence of *S. oleraceus* residue at the rates 150 and 300 g m^{-2} on some soil properties. At the time of harvesting bean, soil samples were collected from each quadrate at 0–30 cm depth to form a one composite sample for each treatment. Residue-free plots were left as control. Values are mean ± standard error of four independent replicates. Means followed by the same lower-case letter in a row do not differ significantly at the 0.05 probability level. EC: electrical conductivity, OC: organic carbon, OM: organic matter.

Soil properties		Rates *of S. oleraceus* residue (g m^{-2} soil)		
		Control	150	300
pH		7.92^{a}±0.01	7.96^{a}±0.02	7.95^{a}±0.04
EC (mS cm^{-1})		0.33^{a}±0.08	0.41^{b}±0.1	0.47^{b}±0.09
OC (%)		1.57^{a}±0.06	1.73^{b}±0.15	1.83^{b}±0.12
OM (%)		2.73^{a}±0.1	3.0^{ab}±0.26	3.18^{b}±0.21
Total phenolics mg/kg soil		31.13^{a}±2.9	195.25^{b}±23.1	273.31^{c}±36.7
Available	N (mg kg^{-1} soil)	98.0^{a}±11.0	138.0^{b}±13.0	152.0^{b}±7.2
	P (mg kg^{-1} soil)	2.61^{a}±0.43	3.25^{b}±0.59	4.48^{b}±0.99
	K (mg kg^{-1} soil)	683.0^{a}±24.0	669.0^{a}±86.0	727.0^{a}±89.0
	Zn (mg kg^{-1} soil)	4.63^{a}±1.05	4.35^{a}±0.88	4.56^{a}±0.48

Table 2. Contents of individual and total phenolic compounds in *S. oleraceus* residue. Phenolic compounds were extracted from dried *S. oleraceus* shoot residue using hydro-acetone, then quantified by HPLC system coupled with diode-array detector. Values are mean ± standard error of four independent replicates.

Compound	Content (μg g^{-1} dry weight)
Caffeic acid	0.32±0.19
Catechol	96.67±37.36
Ferulic acid	104.13±30.45
p-coumaric acid	0.49±0.11
p-hydroxybenzoic acid	2.07±0.80
Resorcinol	31.10±8.70
Sinapic acid	39.70±19.05
Total phenolics	560.5±28.22

Table 3. Shoot growth and yield parameters and concentrations of the different nutrient elements (mg 100 g^{-1} dry weight) measured in *P. vulgaris* as affected by *S. oleraceus* residue at the rates of 150 and 300 g m^{-2}. Bean plants were grown in soils amended with different rates of *S. oleraceus* residues. At harvest, shoot growth and yield parameters were recorded. The produced seeds were analyzed for their minerals using ICP-MS. Values are mean ± standard error of four independent replicates. Means followed by the same lower-case letter in a row do not differ significantly at the 0.05 probability level.

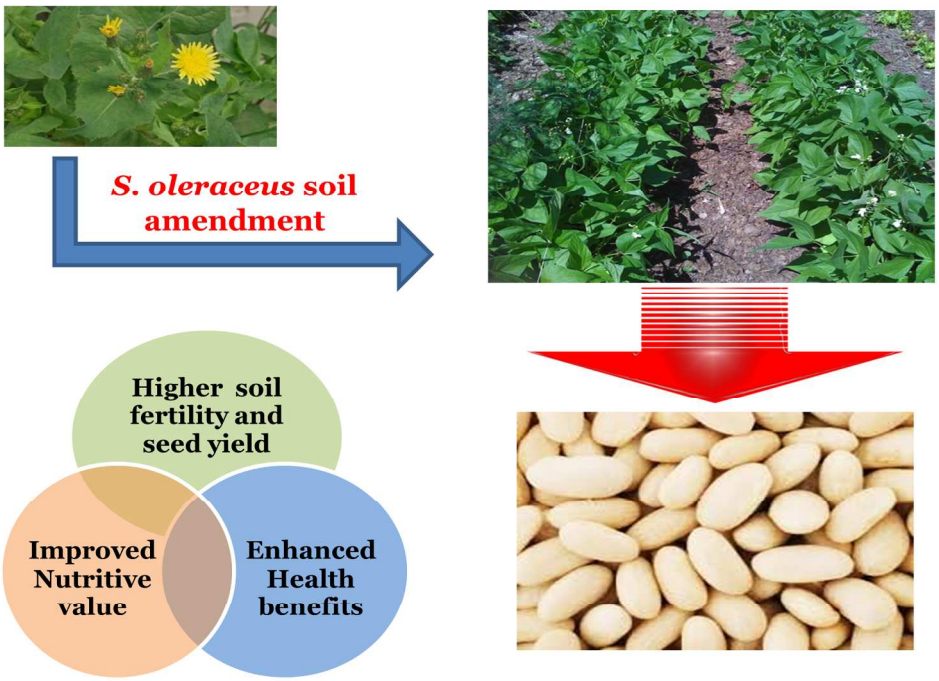
Yield parameter	Rates *of S. oleraceus* residue (g m^{-2} soil)		
	Control	150	300
Shoot growth and yield parameters			
Shoot length (cm)	12.8^{a}±1.8	14.2^{a}±1.3	13.44^{a}±1.5
Shoot biomass (g $individual^{-1}$)	6.5^{a}±1.0	7.5^{a}±0.9	7.6^{a}±0.88
Leaf area $individual^{-1}$ (cm^2)	602.23^{a}±89.5	988.6^{b}±119.7	1141.37^{c}±88.4
Fruit yield (g m^{-2})	831.25^{a}±83.6	1368.5^{c}±122.3	1110.0^{b}±90.08
Pod length (cm)	9.87^{a}±1.1	11.13^{b}±1.2	11.08^{b}±0.79
NO. of pods $individual^{-1}$	19.14^{a}±2.5	20.0^{a}±2.5	20.4^{a}±1.71
NO. of seeds pod^{-1}	5.13^{a}±0.6	6.1^{a}±0.9	5.22^{a}±0.83
Seed yield (g m^{-2})	3220.1^{a}±337.5	5822.2^{b}±598.0	3759.9^{a}±611.7
Nutrient elements			
K	927.0^{a}±13.97	1031.82^{b}±15.55	1330.72^{c}±52.39
Ca	1.13^{a}±0.01	1.27^{ab}±0.11	1.57^{b}±0.14
Mg	119.47^{a}±5.27	135.06^{ab}±6.22	165.86^{b}±10.10
P	161.1^{b}±10.92	220.34^{c}±14.94	114.17^{a}±7.74
Na	13.05^{a}±1.45	18.12^{b}±2.0	17.99^{b}±1.99
Cu	0.98^{a}±0.08	1.14^{ab}±0.09	1.31^{b}±0.11
Fe	0.41^{a}±0.03	0.48^{ab}±0.04	0.55^{b}±0.04
Mn	0.58^{a}±0.04	0.81^{b}±0.05	0.80^{b}±0.05
Zn	2.23^{a}±0.15	3.10^{b}±0.21	3.07^{b}±0.20

Table 4. Concentrations of sugars, organic acids, amino acids and fatty acids measured in *P. vulgaris* seeds in response to *S. oleraceus* residue at the rates of 150 and 300 g m^{-2}. Bean plants were grown in soils amended with different rates of *S. oleraceus* residues. The produced seeds were analyzed for their sugars, organic acids, amino acids and fatty acids using HPLC and GCMS. Values are mean ± standard error of four independent replicates. Means followed by the same lower-case letter in a row do not differ significantly at the 0.05 probability level. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids

Compound	Rates *of S. oleraceus* residue (g m^{-2} soil)			Compound	Rates *of S. oleraceus* residue (g m^{-2} soil)		
	Control	150	300		Control	150	300
Sugars (g 100 g^{-1}dry weight)							
Glucose	7.75^{a}±0.0	7.76^{a}±0.85	6.87^{a}±0.43	Glutamate	1.65^{a}±0.25	1.71^{a}±0.46	2.79^{b}±0.48
Fructose	9.22^{a}±0.49	9.88^{a}±1.09	9.72^{a}±0.87	Glutamine	0.21^{a}±0.05	0.16^{a}±0.003	0.34^{b}±0.003
Sucrose	2.59^{a}±0.0	6.40^{b}±0.063	9.13^{c}±0.17	Ornithine	1.43^{a}±0.14	1.42^{a}±0.25	1.50^{a}±0.16
Total soluble sugars	31.10^{a}±0.64	38.21^{b}±2.60	40.89^{b}±0.96	Total non-essential	2.15^{a}±0.34	2.19^{a}±0.61	3.50^{b}±0.68
Total insoluble	10.88^{a}±0.23	13.38^{b}±0.91	14.51^{b}±0.34	***Total proteins (g 100 g^{-1}dry weight)***	16.13^{a}±2.67	22.47^{b}±1.19	21.15^{b}±2.30
Total sugars	41.98^{a}±0.87	53.36^{b}±1.91	55.20^{b}±1.38	***Fatty acids (mg 100 g^{-1}dry weight)***			
Organic acids (mg g^{-1} dry weight)				Dodecanoic (C12:0)	46.93^{b}±0.0	45.83^{b}±6.60	38.22^{a}±0.0
Citric acid	0.27^{a}±0.06	0.27^{a}±0.08	0.26^{a}±0.06	Tetradecanoic (C14:0)	4.95^{a}±0.11	5.20^{a}±0.012	72.11^{b}±11.29
Fumaric acid	1.13^{a}±0.19	2.14^{b}±0.13	2.41^{b}±0.10	Hexadecanoic (C16:0)	175.22^{a}±24.30	160.05^{a}±24.31	234.36^{b}±62.77
Isobutyric acid	3.30^{b}±0.39	2.62^{ab}±0.50	2.08^{a}±0.01	Heptadecanoic (C17:0)	0.21^{a}±0.005	3.68^{b}±0.086	13.35^{c}±0.57
Malic acid	17.68^{c}±2.94	8.68^{a}±1.44	13.20^{b}±1.80	Octadecanoic (C18:0)	0.041^{a}±0.002	3.56^{c}±0.008	1.99^{b}±0.27
Oxalic acid	1.90^{a}±0.005	1.85^{a}±0.40	1.57^{a}±0.67	Eicosanoic (C20:0)	37.84^{a}±3.26	37.50^{a}±0.08	91.69^{b}±12.25
Succinic acid	0.18^{a}±0.08	0.11^{a}±0.03	0.14^{a}±0.02	Docosanoic (C22:0)	95.45^{b}±10.07	92.10^{b}±13.2	49.93^{a}±7.01
Amino acids (g 100 g^{-1}dry weight)				Tricosanoic (C23:0)	66.17^{b}±4.48	30.25^{a}±0.78	20.957^{a}±2.60
Histidine	0.81^{a}±0.15	1.22^{a}±0.28	1.15^{a}±0.23	Tetracosanoic (C24:0)	0.08^{a}±0.001	0.049^{a}±0.003	0.062^{a}±0.02
Isoleucine	0.21^{a}±0.04	0.35^{a}±0.06	0.45^{b}±0.07	Pentacosanoic (C25:0)	1.17^{b}±0.09	0.47^{a}±0.017	0.43^{a}±0.02
Leucine	2.94^{a}±0.09	2.74^{a}±0.08	3.94^{b}±0.20	*Total SFA*	428.06^{b}±43.91	378.69^{ab}±39.25	523.01^{b}±97.64
Lysine	0.84^{a}±0.17	1.31^{b}±0.15	0.93^{a}±0.26	Hexadecanoic (C16:1, cis-Δ^{7})	283.37^{b}±27.23	323.3^{c}±8.00	233.12^{a}±15.18
Methionine	0.62^{a}±0.15	0.64^{a}±0.19	1.08^{b}±0.26	Octadecenoic (C18:1, cis-Δ^{9})	1.24^{a}±0.03	5.33^{c}±0.36	2.07^{b}±0.35
Phenylalanine	0.88^{a}±0.22	1.73^{b}±0.24	1.06^{a}±0.27	Tetracosenoic (C24:1, cis-Δ^{9})	0.044^{a}±0.004	3.74^{c}±0.08	1.73^{b}±0.23
Valine	0.62^{b}±0.05	0.52^{ab}±0.04	0.26^{a}±0.03	*Total MUFA*	284.65^{a}±29.72	332.37^{ab}±41.38	236.92^{a}±17.29
Threonine	0.23^{b}±0.04	0.21^{a}±0.02	0.46^{c}±0.17	Octadecadienoic (C18:2, cis-$\Delta^{9,12}$)	119.32^{b}±10.80	128.81^{c}±0.67	96.31^{a}±0.26
Total essential	7.16^{a}±0.38	8.71^{b}±0.43	9.34^{b}±0.49	Eicosadienoic (C20:2, cis-$\Delta^{11,14}$)	0.42^{a}±0.01	7.47^{b}±0.17	31.64^{c}±0.62
Non-essential				Hexadecatrienoic (C16:3, cis-$\Delta^{7,10,13}$)	18.19^{a}±1.08	30.06^{b}±2.70	31.67^{b}±0.77
Alanine	0.028^{a}±0.005	0.017^{a}±0.006	0.114^{b}±0.019	Octadecatrienoic (C18:3, cis-$\Delta^{9,12,15}$)	145.18^{b}±9.91	171.35^{c}±13.9	91.57^{a}±10.13
Arginine	0.046^{a}±0.011	0.104^{b}±0.018	0.056^{a}±0.014	*Total PUFA*	283.11^{a}±19.78	337.96^{b}±21.73	251.19^{a}±17.96
Asparagine	0.07^{a}±0.002	0.05^{a}±0.001	0.04^{a}±0.003	*SFA/(MUFA + PUFA)*	0.75^{b}±0.01	0.57^{a}±0.01	1.07^{c}±0.14

Table 5. Contents of free phenolic, flavonoids, vitamins C and E (μg g^{-1} dry weight) and TAC (μmole Trolox g^{-1} DW) in common bean seeds as affected by *S. oleraceus* residue at the rates of 150 and 300 g m^{-2}. Bean plants were grown in soils amended with different rates of *S. oleraceus* residues. The produced seeds were analyzed for their phenolic acids, flavonoids and vitamins using HPLC system coupled with diode-array detector. Values are mean ± standard error of four independent replicates. Means followed by the same lower-case letter in a row do not differ significantly at the 0.05 probability level.

Compound	Rates *of S. oleraceus* residue (g m^{-2} soil)		
	Control	150	300
Phenolic acids			
Caffeic acid	0.192^{a}±0.02	0.26^{b}±0.01	0.96^{c}±0.04
Chlorogenic acid	2.20^{a}±0.29	6.84^{b}±0.08	6.70^{b}±0.32
Ferulic acid	30.13^{a}±3.48	65.20^{b}±1.19	91.75^{c}±4.33
Gallic acid	166.50^{a}±22.15	159.73^{a}±6.60	161.94^{a}±19.49
p-Coumaric acid	35.81^{a}±4.76	50.05^{b}±1.42	109.38^{c}±5.16
Protocatechuic acid	2.45^{a}±1.25	12.17^{b}±0.57	44.30^{c}±2.09
Resorcinol	0.37^{a}±0.04	10.31^{b}±0.71	11.12^{b}±0.55
Syringic acid	9.82^{a}±1.30	18.24^{b}±0.39	30.01^{c}±1.42
Total phenolics	1052.7^{a}±37.23	1292.7^{b}±31.76	1506.0^{c}±43.55
Flavonoids			
Apigenin	2.74^{a}±0.49	4.64^{b}±0.72	2.05^{a}±0.71
Catechin	62.72^{a}±8.34	152.63^{b}±2.49	191.59^{c}±9.05
Luteolin	0.49^{a}±0.07	0.41^{a}±0.02	1.22^{b}±0.08
Quercetin	30.68^{a}±0.35	34.58^{b}±0.53	35.91^{b}±2.34
Rutin	19.98^{a}±0.81	28.88^{b}±0.69	26.65^{b}±2.31
Total flavonoids	656.7^{a}±27.16	739.0^{b}±16.9	756.7^{b}±19.2
Vitamin			
Ascorbic acid	4.20^{b}±0.34	4.98^{c}±0.30	3.17^{a}±0.47
α-tocopherol	8.00^{a}±1.4	11.2^{b}±2.1	12.4^{b}±2.2
β-tocopherol	0.93^{a}±0.20	2.53^{b}±0.59	2.81^{b}±0.51
γ-tocopherol	0.67^{b}±0.08	0.74^{b}±0.11	0.17^{a}±0.06
δ-tocopherol	0.09^{a}±0.03	0.28^{b}±0.06	ND
Total tocopherol	9.79^{a}±1.38	14.75^{b}±1.64	15.38^{b}±2.07
TAC	6.05^{a}±0.25	6.97^{b}±0.19	7.19^{b}±0.43



168x121mm (300 x 300 DPI)