



Paper Details

DOI LINK: <https://doi.org/10.59671/aVWtn>

Paper ID: aVWtn

Volume: 58

Issue: 10

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Keywords: Solanum lycopersicum, growth parameters, rock dust, flavonoids, antioxidant activity, sustainable agriculture

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Effects of basalt flour “Farina di Basalto[®]” on the growth and phytochemical properties of tomato plants

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Abstract

Sustainable agriculture plays an important role in agricultural productivity in that it seeks to reduce chemical fertilizers use and promote the use of alternative, low-cost and environmentally friendly sources. In this sense, basalt dust has shown potential to improve soil fertility and crop nutrition. Thus, an experiment was carried out with the goal to evaluate the potential impact of a basaltic rock flour “Farina di Basalto[®]” type XF on growth parameters of tomato crop under greenhouse. Total leaves content of flavonoids, polyphenols and antioxidant activity were also investigated. Experiments were carried out in a pot system using substrate drenches to apply the basalt flour at different levels, (0%, 1.5%, 3% and 4.5%). The growing media chosen for the trials consisted of a mixture of soil, perlite and potting soil (1:1:1). The results showed that increasing basalt flour to 4.5%, increased significantly growth parameters of tomato plants. However, for plant height and shoot length, increasing dose from 3% to 4.5% was not significant. Results showed

also that leaves dry matter, flavonoids and polyphenols contents were negatively affected when increasing dose to 4.5%. Although, applying of 3% of basalt flour was the most effective in improving antioxidant compounds in tomato plants, the higher antioxidant activity was obtained by applying of 4.5% basalt flour. These results showed the usefulness of basalt flour in improving plant growth parameters and phytochemical compounds offering the possibility of using it as both a natural fertilizer and a biostimulant in sustainable agriculture.

Keywords: *Solanum lycopersicum*, growth parameters, rock dust, flavonoids, antioxidant activity, sustainable agriculture

INTRODUCTION

Mineral nutrition is one of the main levers by which farmers can intervene in order to obtain more abundant production or promote vegetables quality (Fahrurrozi et al., 2016; Kakar et al., 2020). Vegetables require a lot of nutrients and intensive use of the land, a fact that requires the continuous application of fertilizers to maintain productivity. Recently, farmers have become addicted to apply higher doses of inorganic fertilizer. However, excessive use of synthetic fertilizer can lead to nutrient imbalances, groundwater contamination, greenhouse gas emissions hence hampering soil quality, fertility and health (Agbede, 2010; Fahrurrozi et al., 2016; Muktamar et al., 2016; Rahman and Zhang, 2018).

Sustainable agriculture plays an important role in agricultural productivity, in that it seeks to reduce dependence on conventional synthetic fertilizers (Burbano et al., 2022; Dalmora et al., 2022) and promote the use of alternative, low-cost and environmentally friendly sources such as organic fertilizers (Muktamar et al., 2016; Tian et al., 2023) and natural mineral fertilizers by application of rock dust (Burbano et al., 2022; Conceição et al., 2022; Swoboda et al., 2022). In this sense, basalt flour is one of these alternatives, because it is a by-product of rock mining, which can be applied in natural and in addition to being low cost, it has desirable characteristics as an agricultural input, and reduces environmental pollution (Ramos et al., 2020). According to El Desouky (2022), when basalt is crushed to a reasonable size (preferably $\leq 250 \mu\text{m}$) and applied to soils in an

adequate application rate (5 to 20 t ha⁻¹), it acts as a natural fertilizer. It has shown potentials to improve soil fertility (Conceição et al., 2022), crop nutrition and growth under green houses, fields and orchards (Elimem et al., 2020; Melo et al., 2021). Basalt flour is intended for soil mineralization as a source of natural fertilizer (Luchese et al., 2021) due to its rich nutrient contents (Si, Al, K, Fe, Ca, Mg). Conceição et al. (2022) reported that maize and bean plants grown in soils enriched with basalt dust showed macro and micronutrient accumulations, up to five times higher than plants without the use of basalt dust. Some other works showed that basalt flour exerted an effect on pepper crop under greenhouse, improving thus growth parameters such as plant height and leaf area, leaves assimilating pigments rate and fruits sugar content (Rouz et al., 2020). On the other hand, Barak et al. (1983) found that crushed basalt and tuff improved significantly iron nutrition of peanuts plants and their growth on highly calcareous soil and that chlorophyll content was doubled than that in plants grown in untreated soils. The use and incorporation of basalt flour in the soil have grown every year, and there are still no studies showing its effects on the phytochemical properties of plants (under normal conditions) such as phenolic and flavonoid composition and antioxidant activity. The objective of the present work was to evaluate the growth parameters of tomato plants, and to assess the phenolic and flavonoid content of tomato leaves and antioxidant activity, in a pot system after applying different doses of basalt flour using substrate drenches.

MATERIALS AND METHODS

Study location

The experiment took place at the Higher School of Agriculture, situated In Mograne, which belongs to the Zaghouane governorate, located in the north-east of Tunisia (36°25'45.90''N 10°05'36.55''E).

Basalt flour “Farina di Basalto®”

“Farina di Basalto®”: Farina di Basalto® type XF consists of micronized particles ($\varnothing < 20 \mu\text{m}$) obtained through an industrial process. The raw material utilized to

produce “Farina di Basalto®” is sourced from the Castel Viscardo deposit (TR) - Italy. Basalt is an effusive volcanic rock family characterized by a predominantly mafic chemical composition. It is a basic rock devoid of free crystalline silica, amentiferous minerals or other substances harmful to the environment or animal health. Farina di Basalto® is derived through the mechanical grinding of the pure mineral, using ceramic tools, without the addition of other minerals or substances. Furthermore, no materials containing washing water with flocculants, or undesirable and harmful products for agriculture, are used in its production. There are various types of basalt, each with unique characteristics related to their composition, structure and origin. Based on its chemical composition, mineralogy and physical characteristics, this basalt used for the production of Farina di Basalto® (FdB) can be classified as phonolitic tephritic basalt and has unique characteristics. The Basalt used for the production of Farina di Basalto® is particularly prized for its unique composition with micro and lean elements that are useful for plants. Different components of basaltic flour used are shown in Table 1.

Table 1. Physico-chemical characteristics of Basalt flour Farina di Basalto® type XF

	Solid physical state	Flour
	Water solubility	Not soluble in water
	Colour	Slightly gray
	Odour	Not noticeable
	pH	9±0.5 (Log[H+])
	Electrical conductivity (2:1 extract)	1.14 (dS /m)
	Cation exchange capacity	9 (meq/100g)
	Assimilable iron (As. Fe)	377 (mg/kg)
	Density	2.70 (kg/dm ³)
	SiO ₂	47%
	K ₂ O	9%
	Fe ₂ O ₃	6.85%
	CaO	8%
	MgO	2.25%
	Na ₂ O	3.55%
	P ₂ O ₅	0.65%
	TiO ₂	0.6%
	Mn	636 mg/Kg
	S	536 mg/Kg
	B	81 mg/Kg
	Cu	51 mg/Kg
	Zn	68 mg/Kg

Trial essay

The experiment was carried out in a 90 m² greenhouse. Pots with 5 L soil capacity were used and filled with a growing media consisting of a mixture of peat, perlite and local soil (1:1:1). The tomato variety « Firenze » was planted on March 13, 2023 at the 3-leaf stage. Growth parameters and phytochemical properties of tomato plants were evaluated after addition of different amounts of basalt flour. Treatments consisted of four concentrations (0%, 1.5%, 3%, and 4.5%) of basalt flour applied as a solution around the stem of the plants (substrate drenches), with the first application being administered 3 days after transplanting. In total, three applications were made at intervals of 3 weeks at the rate of 100 ml/plant. Plants were monitored and irrigated three times a week with tap water throughout the experiment to maintain optimal soil moisture. Neither fertilizers nor pesticides were applied. The essay was conducted twice and established according to a complete random arrangement with six replications for each treatment.

Growth analysis of tomato plants

The experiment was concluded at 12 weeks after transplantation. Plant height (cm) was measured from the crown to the highest point using a measuring tape and stem diameter (mm) at 10 cm from the substrate surface was also measured using caliper. After measuring height and diameter, plant shoot and roots, already washed under tap water, were weighted for fresh weight. Dry matter of leaves was measured after drying at 60°C for 72 hours (Conceição et al., 2022). Root length was determined and Leaf area was estimated using the “Mesurim pro software (2019)”. For this, leaves were sampled from median part of the plant at the rate of three leaves/plant/treatment.

Phytochemical parameters of tomato plants

Preparation of the plant extracts

Plant leaves were separated, placed in paper bags and then in a forced-air circulation oven, where they remained at 60°C for 72 h. After drying, they were ground to flour

before analysis. 10 grams of flour were mixed with 100 mL of distilled water. After filtration, each extract was stored at 4°C prior to evaluate the antioxidant activity and quantitation of phenolics and flavonoids.

Determination of Total Phenolic and Flavonoid Contents

Total phenolic content

The determination of total phenolic content of the different plant extracts was carried out, according to Folin-Ciocalteu procedure, by using the method of Chetoui et al. (2013). 0.5 mL of diluted sample was added to 2 mL of Folin-Ciocalteu reagent. A volume of 2.5 mL of Na₂CO₃ (7.5%) was added, after incubation for 5 mins. The absorbance at 760 nm was read, after incubation for 90 min. The total phenolic content was calculated as gallic acid equivalent (GAE) in mg/g dry weight of extract (DW). It was expressed in mg GAE/g DW.

Total flavonoid content

The determination of total flavonoid content was carried out by using the method of Chetoui et al. (2013). 1 mL of diluted extract was mixed with 1 mL of 2% AlCl₃. The absorbance was measured at 430 nm, after incubation for 15 min. The percentage content of flavonoids was expressed as rutin equivalent (RE) mg/g dry weight of extract (mg ER/g DW).

Antioxidant activity

The antioxidant activity of tomato plants was evaluated by using 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity, as reported by Zaouali et al. (2010). 3 ml of DPPH (4x10⁻⁵ M) was added to 1 ml of diluted extracts. The absorbance was measured at 517 nm after incubation for 30 mins. Trolox was used as positive control. The capability to scavenge the DPPH radical was calculated, using the following equation:

$$\text{DPPH scavenged (\%)} = \{(Ac - At)/Ac\} \times 100$$

Where Ac is the absorbance of the control reaction and At is the absorbance in presence of the sample of the extracts.

Statistical analysis

The experimental design used was randomized blocks with four treatments and six replications. All analyses were performed in triplicate and the results were reported as means of three measurements. An analysis of variance (ANOVA) was performed on the data using the SASS statistical program (version 20). Mean comparison was performed by Duncan Test at 5% level.

RESULTS

Basalt effect on tomato plants growth

Plant height

Plant growth of different treatments (basalt doses) was investigated 12 weeks after transplanting. As illustrated in Table 2, growth parameters of tomato plants, using basalt flour in substrate drenches, were higher than those recorded in the control (0% basalt). In fact, addition of basalt flour increased plant height, which was improved significantly with the increased levels of basalt flour. During this study period, maximum height registered was about 40.33 cm for T2 (3%). The same Table indicates that the application of basalt above 3% retarded plant height but statistically, the use of 3% (T2) or 4.5% (T3) of basalt produced no significant difference in plant height.

Table 2. The averages for height, root length, stem diameter and leaf area of tomato plants 12 weeks after transplanting.

Tomato growth parameters				
Treatments	Plant height (cm)	Stem diameter (mm)	Root length (cm)	Leaf area (mm ²)
Control (0%)	30.33c	5.27d	27.80b	31.94d
T1 (1.5%)	35.66b	6.28c	30.80b	38.59c
T2 (3%)	40.33a	6.58b	36.60a	47.43b
T3 (4.5%)	38.66a	7.50a	38.60a	58.83a

Means not followed by the same letters within a column differ significantly by the Duncan test at the 5% error probability level.

Stem diameter

Observing the results for stem diameter, there was a statistically significant difference ($p < 0.05$) among treatments (Table 2). Increasing dose of basalt flour in the irrigation water increased plant stem diameter from 5.27 mm (0%) to 7.5 mm in the treatment T3 (4.5%).

Root length

The effect of varying basalt flour doses on root length of tomato was significant in this study. Tomato plants grown in medium enriched with basalt flour showed root length longer than plants without basalt flour (Table 2). The highest value (38.6 cm) was observed with the dose of 4.5% basalt application. There was a statistically significant difference between the control and doses (T2 and T3) which showed the higher values. Use of 3% or 4.5% of basalt didn't show a significant difference on root length. Data from the same table reveal also that there is no significant difference between T1 (1.5%) and control 0%.

Leaf area

Data from Table 1 reveal that leaf area was affected positively by basalt flour application. Values dropped from 31.94 mm² in control samples to 58.83 mm² in plants irrigated with high level of basalt flour T3 (4.5%). Obtained results showed a significant difference between treatments at $p \leq 0.05$ (Table 2).

Shoot fresh weight (g)

In Table 3, it can be seen that application of basalt flour produced a higher plant fresh weight compared to the control. As illustrated, the averages found for plants shoot fresh weight showed that all treatments were superior to the control. Treatment T3 (4.5%) best promoted shoot biomass with a weight of 29.3 g, while the weight for control was 7.12 g. According to the same table, treatment T1 produced no significant difference in shoot weight (20.32 g) compared to T2.

Table 3. Effect of different treatments on biomass (g) of tomato plants 12 weeks after transplanting

Tomato growth parameters			
Treatments	Shoot fresh weight(g)	Root fresh weight (g)	leaves dry matter (%)
Control (0%)	7.12 c	9.20 c	23 b
T1 (1.5%)	20.32 b	26.37 b	29.60a
T2 (3%)	21.05 b	33.15 ab	31.76a
T3 (4.5%)	29.30 a	40.56 a	25.45b

Means not followed by the same letters within a column differ significantly by the Duncan test at the 5% error probability level.

Root fresh weight

According to Table 3, root fresh weight reached its maximum with T3 (40.56 g). However, the least value was founded in control (9.2 g). Data from this table showed that basalt flour application had a high impact on root fresh weight. In fact, tomato plants showed a quadratic increase in root fresh mass with T3 (4.5%). A statistically significant difference was noted between the control and the basalt treated plants. However, treatment T2 produced no statistical difference in root weight (33.15g) compared to T1 (26.37g) and T3 (40.56g).

Leaves dry matter

The maximum average value was observed for T2 with 31.76% followed by T1 with 29.60% and lowest dry matter value was recorded for control with 23%. It should be noted that increasing basalt flour dose to 4.5% had slightly increased leaves dry matter compared to control (Table 3). Difference between treatments T1 and T2 is statically insignificant at $p \leq 0.05$. In addition, no effect of the dose of 4.5% of basalt was observed compared to control plants.

Basalt effect on tomato phytochemical parameters

Total Phenolic and Flavonoid Contents

The results of the investigation showed that tomato plants, treated by basalt are rich of polyphenols and flavonoids than the control (Table 4). The same table shows that

treatment T2 (3%) exhibited the best contents of both polyphenols and flavonoids (0.497mg GAE/g DW and 0.934mgRE/g DW respectively). Statistically, this study showed no significant difference between control (0%) and the higher dose of basalt (4.5%)

Table 4. Basalt effect on tomato phytochemical parameters

Phytochemical tomato plants contents			
Treatments	Polyphenols (mg GAE/g DW)	Flavonoids (mg RE/g DW)	IC50 (µg/ml)
Control (0%)	0.284 b	0.893 b	413.784a
T1 (1.5%)	0.259 b	0.832 b	376.809b
T2 (3%)	0.497 a	0.934 a	282.423c
T3 (4.5%)	0.310 b	0.910 ab	232.479d

Means not followed by the same letters within a column differ significantly by the Duncan test at the 5% error probability level.

Antioxidant activity

The antioxidant capacity of tomato leaves was evaluated by the DPPH scavenging assay. According to Table 4, the antioxidant behavior was increased by increasing the concentration of basalt flour from 0% to 4.5%. Treatment T3 (4.5%) revealed the highest scavenging activity by showing the lowest IC50 (232.479 µg/ml) followed by T2, T1 and control, respectively. The lowest DPPH scavenging activity was observed in the control with very high IC50 value of 413.784µg/ml.

DISCUSSION

Inorganic fertilizers are the mainstays of conventional crop management strategies, which have enhanced agricultural production (Li and Dong, 2013; Kouyate et al., 2023). Yet, using too much chemical fertilizer can have a harmful impact on the environment in addition to not promoting plant growth (Li and Dong, 2013; Naher et al., 2019). In addition, their long-term impacts on soil organic carbon pools and soil physical attributes are not fully understood (Canqui and Schlegel, 2013). According to Mäder et al. (2002), their use may accelerate the breakdown of organic matter and perhaps lessen aggregate stability. Therefore, keeping soil quality is an

essential requirement for sustainable agriculture (Asghar et al., 2022). The search for higher yields, lower production costs, and increased sustainability in agriculture implies optimizing crop nutritional management (Conceicao et al., 2022). Actually, rock dust gains momentum due its beneficial spin-offs compared to conventional marketed fertilizer.

In the present study, the potential impact of basaltic rock flour at different levels of applications, on tomato plant growth, was assessed. For plant height, stem diameter, root length and leaf area of tomato plants, there was a significant effect in response to soil drenches of basalt flour. There was a tendency of increase in plant growth parameters with increasing doses of basalt flour from 1% to 4.5%. These results agree with those of Dalmora et al. (2022) whose work with basalt dust showed an increase on the height and stem diameter of tomato plants. Similar results were observed by Conceição et al. (2022), who found an increase in height and stem diameter of maize and bean plants after incubating soil with basalt dust. Melo et al. (2021), also observed that the use of basalt dust or powder increased growth of murici (*Byrsonima crassifolia*).

The best results obtained with the use of basalt flour can be attributed to the nutritional composition of the rock flour itself (macro and micronutrients). In fact, basalt flour wastes from mining activities, is among the most studied rock flours; it showed the potential to be used as a natural fertilizer (Krahi et al., 2020) and it is evaluated as low-cost indigenous nutrient sources which is able to supply nutrients required for optimal crop growth (Burbano et al., 2022; Dalmora et al., 2022; Luchese et al., 2023). According to Ramos et al. (2020) rock dust is an agricultural input that contains essential macro and micronutrients for plants. In our case, increasing growth parameters cited previously, can be attributed especially to the richness of the used basalt flour on Silicon ($\text{SiO}_2=45-49\%$, Table 1). Although this element is not considered an essential element for most plants, ample evidence is presented that silicon, when readily available to plants, plays a large role in their growth and their mineral nutrition (Costa et al., 2016). Results found by Souza Costa et al. (2016), showed that increasing silicon concentrations provide good growth in passion fruit plants and conduce to an increase in the stem diameter

and plant height. However, a reduction in those parameters can occur at higher concentrations of Silicon, according to the same authors. For plant height and root length, there was no significant effect in response to increasing basalt dose and use of this fertilizer at a dose of 3% is as effective as at 4.5%. Whereas, increasing basalt flour to 4.5% increased significantly leaf area and stem diameter. Similar results were found by Rouz et al. (2020) when they applied two doses of basalt flour (1% and 3%) on pepper. The authors observed an increase on growth parameters such as plant height and leaf area. They concluded that, use of the fertilizer at a dose of 1.5% is as effective as at 3%. Increasing basalt concentrations resulted in a proportional increase in the fresh shoot and root biomass, reaching their maximum with the higher basalt dose (4.5%). These results agree with those of Dalmora et al. (2022) who showed that the application of amygdaloidal basalt in the soil increased green and dry mass of tomato shoots and roots.

Concerning dry matter, results obtained in this study indicated that the application of basalt flour increases the leaves dry matter but at the higher concentration (4.5%), a downward trend in values was recorded. In their study, Rouz et al. (2020) showed, in the contrary, that basalt has no effect on the dry matter contents of different parts of the pepper plants. This is probably due to the increasing of silicon concentration in the basalt solution. In fact, Costa et al. (2016) observed a linear decrease for dry biomass with the increasing of silicon concentrations. Despite increase in leaf area with 4.5% of basalt, the same dose didn't improve leaves dry matter. According to Gomes et al. (2008), an increase in the leaf area does not always mean an increase in the amount of dry matter produced by the plants. The results obtained in this article show that and they are similar to those obtained by Costa et al. (2016). Tomato plants grown in substrates treated with basalt flour were significantly vigorous, suggesting that, even in a short period of interaction of this material with the substrate (less than 90 days), there was surly an improvement in characteristics of this substrates, which resulted in the greater development of tomato plants (Luchese et al. 2021). However, Asridawati et al. (2021) have another point of view; their work showed that the effect of basalt on plant growth (corn) was not significant because the nutrients derived from this silicate rock dust were

very slowly available. Thereby, to be efficient, basalt rock dust should be treated by heating to a temperature of 700°C with the solvent peat humic.

While the effect of rock dust and particularly basalt flour was widely studied, there is no information available on their effect on phytochemical characteristics to date. Thereby, this present study is a preliminary essay to evaluate the impact of basalt flour on antioxidant activity, flavonoids and polyphenols contents of tomato plants. Results showed that addition of basalt flour to plants improved their contents in polyphenols and flavonoids. The dose of 3% was the most effective dose. Hence, increasing dose to 4.5% had no significant difference with nontreated plants. This study also showed that the antioxidant activity (evaluated by the DPPH scavenging assay) was increased by increasing the concentration of basalt flour from 0% (IC₅₀=413.784µg/ml) to 4.5% (IC₅₀ =232.479 µg/ml). Photochemical parameters of plants refer to the various chemicals compounds found in plants that have potential health benefits or biological activities. Basalt used in this study can potentially enhance the antioxidant activity of plants probably by providing essential nutrients such as Ca, Mg, K and especially Silicon (45-49%). In fact, some researchers suggested that rock content silicon may enhance antioxidant activity in plants. Abd-Alkarim et al. (2017), found that both foliar and soil silicon treatments, by using of diatomite (rock containing 86-89% of SiO₂) significantly increased the ascorbic acid content in Cucumber plants. Silicon (Si) is a nonessential element for plant growth, but it influences the tolerance to biotic and abiotic stresses in many plant species (Silva et al., 2022). In response to soil drenches of basalt flour, tomato plants increased the synthesis of polyphenols and flavonoids, these compounds will protect them from biotic stress like pests and diseases. In fact, several studies showed the beneficial effect of rock dust and basalt in reducing pests (Li and Dong, 2013; Farone et al., 2020; Limem et al., 2020). Further researches should be realized to ensure these results.

Conclusion

Basalt flour (Farina di Basalto) applied on tomato plants under greenhouse conditions with four different doses (0%, 1.5%, 3% and 4.5%), in substrate drenches, had a significant influence on growth parameters of tomato such as

height, stem diameter, leaf area and green fresh weight. Phytochemical parameters, such as flavonoids and polyphenols were also improved compared to plants without basalt flour. However, for plant height and shoot length, increasing dose from 3% to 4.5% was not significant. Leaves dry matter, flavonoids and polyphenols contents were negatively affected when increasing dose to 4.5%. In general, it has been shown that the 3% dose of basalt flour allowed to obtain a high concentration of antioxidant compounds (flavonoids and polyphenols), hence the higher antioxidant activity was obtained by applying of 4.5% basalt flour. Therefore, the availability and diversity worldwide of by-products originating from the extraction and processing of rock have the possibility of becoming an alternative to chemicals fertilizers, and a novel way for enhancing plants tolerance to biotic stress, with low costs and low environmental impact.

ACKNOWLEDGEMENTS

Authors would like to express their profound appreciation to Dr. Slim ROUZ and Dr. Mohamed ELIMEM for providing all the necessary laboratory materials for phytochemicals analyses.

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