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Research Article

Effect of NPK fertilizer on the biochemical response of tomatoes (*Solanum lycopersicum* L.)

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Abstract

Increasing nutrient inputs affect plants and soil. Long-term, repeated mineral fertilizer applications may alter the agro-ecosystem. The application of the right amount of fertilizer is primordial to maintaining and improving sustainable plant productivity. The physiological response of the plant provides meaningful information on yield and makes it possible to better choose the doses of fertilization amendments. In order to determine the adequate dose of NPK fertilizer under economically profitable and more environmentally sustainable conditions, we investigated the impact of the chemical fertilizer amended at different NPK fertilizer doses (15, 20, and 25 kg ha^{-1}) on the growth parameters, oxidative metabolism, and yield of *Solanum lycopersicum*. A complete random block experimental set-up with three doses and four replicates was performed for a greenhouse tomato crop. The results showed that NPK fertilizer influences morphological parameters, and phenolic compounds with the best data correlated to the medium treatment. Likewise, the content of flavonoids, lycopene, and β-carotene in tomato fruits displays a similar trend of variation with all treatment doses. The biochemical responses of plants to mineral fertilizer indicate that a medium dose is suitable, without overuse of fertilizers and pesticides, to reduce the negative impact on the agro-ecosystem.

Keywords: agro-ecosystem; oxidative stress; phenolic compounds; plants; soil

Introduction

Population growth, food insecurity, and the reduced fertility of agricultural soils are leading to an increased use of chemical fertilizers. It is one of the most important factors that help to enhance plant production; it is represented as a nutrient source for plants that can be included in the soil to boost its productivity (Kwon *et al*., 2019). The use of a drip irrigation system, as well as the application of recommended doses of mineral fertilizer N-P-K and phytosanitary treatments, improves tomato yield and quality.

Nitrogen, phosphorus, and potassium (NPK) are essential elements for the optimum growth and development of plants. Nitrogen is a major element in protein, nucleic acid, enzymes, and chlorophyll

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(Koeduka *et al*., 2006), in the case of a deficiency, leaves show signs of chlorosis. The second significant nutrient is potassium, which is mostly found in plants at an important concentration. The perturbation in nitrogen metabolism due to potassium deficit occurs due to modifications in the ratios of nitrogen factions, an accumulation of ammonium ions, and toxic amino compounds (agmatine, N-carbamoyl putrescine, and putrescine) (El Gendy *et al*., 2015).

Potassium is essential for maintaining osmotic potential in cells. It's summarized in the activation of an enzyme involved in respiration and photosynthesis in the youngest leaves; thus, the deficiencies appear mainly on the older leaves. Potassium deficiency is characterized by mottled or marginal chlorosis, and the roots are very sensitive to root rotting fungi present in the soil. Phosphorus is also an important macroelement for plant growth and development and exists in sufficient concentration in plant tissues. Phosphorus is considered the major element in respiration and photosynthesis (Denison and Kiers, 2005). It is the primary component of proteins, lipids, and nucleic acids, and a lack of it slows the growth of young plants and causes necrotic spots, which are dark green blotches on the leaves.

The application of the right amount of fertilizer is primordial, finding the perfect balance between nutrient availability, fertilizer dose, and application time is a crucial parameter to improve fertilizer's performance and prevent environmental contamination. Excessive amounts of fertilizer create an abiotic stress on the plant, and since tomatoes are a highly strategic crop, growers aim to overuse fertilization. Studies have shown that fertilization changes plant biochemical contents, which affect plant growth and productivity (Wang *et al*., 2001). In normal conditions, plants produce reactive oxygen species (ROS) for a normal growth and development. ROS also acts as a signal molecule at a low level in order to help plants tolerate biotic and abiotic stresses (Hasanuzzaman *et al*., 2011). In order to eliminate the deleterious effects of the over-generated ROS, plants have developed non-enzymatic and enzymatic antioxidant pathways.

The antioxidant system includes reduced glutathione, ascorbate, tocopherol, flavonoids, and carotenoids, while enzymatic ROS scavenging mechanisms include catalase, superoxide dismutase, ascorbate peroxidase, glutathione peroxidases, glutathione S-transferases, and glutathione reductase.

Tomatoes are one of the most consumed vegetables in Algeria and are considered a strategic crop. It is an excellent source of vitamins, minerals, and antioxidants, and its uptake has been linked with a diminished risk of chronic degenerative diseases (Ngcobo *et al*., 2020). Vitamin A, folate, ascorbic acid, and potassium are among the nutrients found in substantial amounts in tomatoes. Carotenoids (lycopene, zeaxanthin, and β-carotene) are all present, as are glycoalkaloids (tomatine), phenolic acids, and flavonoids. These bioactive components give the tomato several biological virtues, such as antioxidant activity, anti-inflammatory, anti-mutagenic, and anti-atherogenic properties (Chaudhary *et al*., 2018).

To estimate the appropriate NPK dose for tomato growth under commercially viable and ecologically acceptable conditions, we tested three doses of mineral fertilizer (NPK) (15, 20, and 25 kg ha^{-1}). Our hypothesis was to confirm whether the dose used by most farmers in Algeria gives the best yield in terms of agriculture, economy, and health level to prevent those who aim to overuse fertilizers. Growth parameters, oxidative metabolism, and yield were evaluated to see the impact of these mineral inputs on the physiological response of the cultivated tomato.

Materials and Methods

Culture, condition of the plant, and treatment

The study was conducted on an experimental farm in Mazagran, Mostaganem (west Algeria). The tomato plants (*Solanum lycopersicum* L., variety 'Belfast F1') were planted by hand in mid-February 2019 and harvested in June 2019 at maturity. The NPK fertilizer 20.20.20 (composition: total nitrogen (N), 20%; phosphoric anhydride (P_2O_5), 20%; potassium oxide (K_2O), 20%; sulfuric anhydride (SO_3), 1%; magnesium

oxide (MgO), 1%) used was manufactured by Profert (Algeria). Fertigation is the mode of application of this water-soluble NPK ternary fertilizer. A complete random block experimental set-up with three doses and four replicates was carried out for a greenhouse tomato crop. The doses of NPK tested are 15, 20, and 25 kg ha^{-1} in order to determine the most suitable dose for tomato cultivation and to check whether the dose recommended by the Algerian Ministry of Agriculture is the best. Therefore, zero control was not applied here. All the experimental conditions have been applied and respected, such as irrigation and phytosanitary treatments. The leaves were harvested at two stages of the plant's growth, initial stage of growth and the final stage of maturation. They were stored in the laboratory at 4 °C in order to perform the biochemical analysis.

Particle size distribution and chemical characteristics of soil samples

After oxidizing organic matter with 5% H_2O_2 and physically dispersing it with ultrasonic, the particle size distribution was measured using a laser granulometer (Muggler *et al*., 1997). The pH in water, Al, Mg and Ca contents, and electrical conductivity were determined by the method of Tedesco *et al*. (1995). Total organic carbon (TOC) and total nitrogen (TN) quantification was performed by dry combustion using the Flash EA 1112 elemental analyzer (Thermo Finnigan, Milan, Italy). Soil organic matter (SOM) content was estimated by multiplying the TOC content by the factor 1.724

Measurement of vegetative growth parameters

The vegetative growth of tomatoes was estimated by measuring leaf area (cm^2), plant height (cm), tomato fruit number, tomato weight (kg) per plant, and total yield per hectare.

Analysis of photosynthetic pigments

The photosynthetic pigments were determined using the protocols and formulae of Lichtenthaler and Buschmann (2001).

Estimation of phenolic contents and flavonoid measurement

The method of Singleton and Rossi (1965) was used to determine the total phenolic content. Flavonoids in the extracts were determined using the protocol given by Zhishen et *al*. (1999).

Activity of antioxidant enzymes

In 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, and 1% polyvinylpolypyrolidone (PVP) were used to extract enzymes from fresh leaves (0.3 g). The protein content was determined by the protocol of Bradford (1976), and the antioxidant enzyme activity (catalase, APX, and PAL) was measured by Aebi (1984), Nakano and Asada (1981), and Beaudoin-Eagan and Thorpe (1985), respectively.

Antioxidant capacity: DPPH scavenging activity and FRAP assay

The scavenging activity of the diphenylpicrylhydrazyl radical (DPPH) was evaluated according to Shimada *et al*. (1992). The FRAP assay was determined by the method of Rosales *et* al. (2006).

Determination of malondialdehyde (MDA) content and hydrogen peroxide (H_2O_2) level

Estimation of Lipid peroxidation is established by the method of Health and Packer (1968), and the accumulation of H_2O_2 in leaves according to Velikova *et al*. (2000) method.

Measurement of proline, tocopherol, pigments, and soluble sugar content

Proline concentration was estimated using the method of Bates *et al*. (1973), and the estimation of tocopherol level followed Martinek's (1964) method. Lycopene and β-carotene concentrations were quantified

according to the method of Nagata and Yamashita (1992). The soluble sugar content is determined by the method of Dubois *et al*. (1956).

Statistical analysis

The results were expressed as mean ± standard deviation. An analysis of variance was made by the SPSS package (IBM SPSS Statistics, version 24.0). The value of $p < 0.05$ represents a significant difference.

Results

Soil properties of the experimental plots

According to soil samples analysis, the texture class of the cultivated soil was loamy sand, characterized by very low organic matter content (SOM) and low electrical conductivity (EC) indicating non-saline soil (Table 1). In correlation with the SOM, the total organic carbon and the total nitrogen contents were very low. The concentrations of exchangeable cations Ca^{+2} and Mg^{+2} are low, with the predominance of Ca^{+2} reflecting the limited capacity of cultivated soils to retain these cations. Aluminum is absent, which confirms the non-toxicity of these soils.

Table 1. Particle size distribution and chemical characteristics of samples

Samples	Sand	Silt	Clay[1]	pH H_2O	Al	Mg	Ca	EC[2]	TN	TOC	SOM[3]
	—— % ——			01:01	$cmol_c\ dm^{-3}$			$\mu s\ cm^{-1}$	—— $g\ kg^{-1}$ ——		%
T1	78.0	12.2	9.8	7.7 ± 0	0	0.2±0	5.8±0	741.7 ± 0	0.928±0	9.055±0	1.56± 0
T2	78.45	13.15	8.4	7.8 ± 0.14	0	0.2±0	5.5± 0.14	435.8 ± 122.3	0.683± 0.004	6.262 ± 0.068	1.08 ± 0.01
T3	77.15	12.25	10.6	7.85 ± 0.21	0	0.2±0	5.3±0.28	527 ± 289	0.931 ± 0.137	8.586 ± 1.635	1.48 ± 0.28

[1]Sand: >0.2 mm, Silt: < 0.2 and >0.05 mm, Clay: <0.05 mm.

[2] pH: hydrogen potential, Al: Aluminum, Mg: magnesium, Ca: calcium and EC: electric conductivity.

[3]TN: Total Nitrogen; TOC: Total Organic Carbon, SOM: Soil Organic Matter

Effect of NPK fertilizer on the growth and yield of tomatoes

The effect of different doses of NPK fertilizer on vegetative growth and yield of tomatoes was evaluated (Table 2). The vegetative growth of tomato was significantly influenced by growth stage ($p<0.05$). The maximum tomato fruit number per plant and yield were obtained with T3 application compared to other treatments.

Table 2. Effects of NPK fertilizer on leaf area (cm^2) for the initial and final growth stages, plant height (cm), tomato fruit number/plant, tomato weight per plant and total yield/ha of tomato plants

Treatment	Initial growth stage	Final growth stage		Tomato fruit number per plant	Tomato weight per plant (Kg)	Total yield per ha (ton)
	Leaf area (cm^2)	Leaf area (cm^2)	Plant height (cm)			
T1	119.73 ± 71.58[a]	390 ± 140[b]	123 ± 20[a]	18.03 ± 3.42[a]	1.65 ± 0.50[a]	35.62 ± 0.50[a]
T2	191.14 ± 72.23[a]	563 ± 187[b]	140 ± 16[a]	21.10 ± 5.72[a]	2.25 ± 0.56[a]	47.42 ± 0.56[b]
T3	222.87 ± 56.99[a]	510 ± 132[b]	137 ± 17[a]	25.07 ± 5.66[b]	2.55 ± 0.50[a]	53.75 ± 0.50[c]

Treatments: T1, 15 kg NPK ha^{-1}; T2, 20 kg NPK ha^{-1}; T3, 25 kg NPK ha^{-1}

Values followed by different small letters are significantly different at $p< 0.05$

Effect of NPK fertilizer on chlorophyll, carotenoids, phenolic compound content, and flavonoids

Chlorophyll content is often used to evaluate the impact of many environmental stresses. The application of the NPK fertilizer had no significant effect on the content of chlorophyll (a) and (b) in both stages (Table 3). Total chlorophyll showed a significant difference in the initial growth stage, with the highest content in T2 and the lowest in T1, followed by T3. However, the final growth stage did not show a significant difference, with the highest content in T2 and less in T3, followed by T1. Carotenoids showed no significant difference in the initial growth stage, with the highest content using treatment T1, followed by T2, and T3, whereas in the final growth stage, a significant difference has been recorded, with the highest value in T1, followed by T3, and finally T2. Phenolic content showed no significant difference between treatments, whereas there was a significant difference between the two stages. The flavonoid content showed no significant difference between NPK doses, but a significant difference was found between the two growth stages (Table 3).

Table 3. Effects of NPK fertilizer on contents of chlorophyll a, b, total chlorophyll, carotenoids, phenolic compound content, tocopherol, proline contents, malondialdehyde (MDA), hydrogen peroxide levels, free radical scavenging capacity (DPPH), and FRAP activity of tomato plants

Parameters	Initial growth stage			Final growth stage		
	T1	T2	T3	T1	T2	T3
Chlorophyll ***a*** ($\mu g.g^{-1}FW$)	171 ± 31^{a}	159 ± 31^{a}	137.16 ± 17.19^{a}	789 ± 148^{b}	799 ± 173^{b}	677 ± 158^{b}
Chlorophyll ***b*** ($\mu g.g^{-1}$ FW)	111 ± 25^{a}	143 ± 40^{a}	88.55 ± 9.55^{a}	451 ± 43^{b}	605 ± 145^{b}	649 ± 135^{b}
Total chlorophyll ($\mu g.g^{-1}$ FW)	282 ± 53^{abc}	309 ± 69^{b}	225 ± 24^{c}	995 ± 182^{d}	1479 ± 230^{d}	1113 ± 106^{d}
Carotenoids ($\mu g.g^{-1}$ FW)	52 ± 16^{a}	40 ± 13^{a}	46 ± 12^{a}	241 ± 43^{b}	173.07 ± 9.73^{c}	209 ± 23^{b}
Flavonoid (μg QE/g FW)	69 ± 11^{a}	92 ± 15^{a}	64 ± 31^{a}	233 ± 74^{b}	284 ± 73^{b}	349 ± 55^{b}
Total phenol (mg GAE/g FW)	3.95 ± 0.49^{a}	05.06 ± 1.50^{ab}	3.90 ± 0.64^{a}	6.7 ± 0.91^{b}	6.87 ± 1.24^{b}	6.56 ± 1.24^{b}
Tocopherol (μM/g FW)	0.35 ± 0.10^{a}	0.30 ± 0.05^{a}	0.33 ± 0.13^{a}	1.46 ± 0.20^{b}	1.09 ± 0.57^{b}	1.66 ± 0.33^{b}
Proline (M/g FW)	3.69 ± 0.74^{a}	7.76 ± 1.72^{b}	6.20 ± 1.22^{c}	3.06 ± 0.48^{a}	3.45 ± 1.07^{a}	2.69 ± 0.43^{a}
MDA (μM/g FW)	0.09 ± 0.01^{a}	0.11 ± 0.01^{a}	0.11 ± 0.02^{a}	0.23 ± 0.07^{b}	0.37 ± 0.07^{b}	0.26 ± 0.01^{c}
Hydrogen peroxide (μM H_2O_2/g FW)	1.2 ± 0.20^{a}	1.45 ± 0.35^{ab}	1.72 ± 0.30^{b}	2.19 ± 0.12^{c}	2.31 ± 0.34^{c}	2.35 ± 0.28^{c}
DPPH inhibition (%)	12.28 ± 5.69^{a}	18.68 ± 4.53^{a}	12.5 ± 7.2^{a}	63.68 ± 6.94^{b}	64.22 ± 5.83^{b}	63.68 ± 7.41^{b}
FRAP activity (μM ferrous equivalent)	41.71 ± 20.27^{a}	46.87 ± 12.56^{a}	45.25 ± 17.64^{a}	398 ± 53^{b}	389 ± 47^{b}	377 ± 33^{b}

Treatments: T1, 15 kg NPK ha^{-1}; T2, 20 kg NPK ha^{-1}; T3, 25 kg NPK ha^{-1}; μg QE/g FW, μg of quercetin equivalent per gram of fresh weight; mg GAE/g FW, mg of gallic acid equivalent per gram of fresh weight. Values followed by different small letters are significantly different at $p < 0.05$

The tomato is an important source of ascorbic acid and tocopherol. In the present study, no significant changes were observed in tocopherol contents during NPK treatment and growth stages ($p > 0.05$) (Table 3). Proline has a role in enhancing the antioxidant system and fighting stress damage. Its accumulation is also a true stress tolerance mechanism. The effect of the NPK fertilizer on proline is shown in Table 3. A significant

difference in proline content as influenced by NPK treatments and growth stage was observed, whereas no significant difference was found in the final growth stage.

Effect of NPK fertilizer on MDA and H_2O_2

The results of MDA showed no significant difference between the three treatments: T1, T2, and T3 in both stages (the initial and final growth stages) (Table 3). Hydrogen peroxide (H_2O_2) is a strong oxidizing agent. However, an excessive accumulation of H_2O_2 is responsible for oxidative stress; no significant difference was found between the three treatments.

Effect of NPK fertilizer on DPPH and FRAP activity

DPPH free radical scavenging potential showed a non-significant change in the leaves harvested from treatments T2 and T3 supplemented with 20 and 25 kg NPK ha^{-1} in both initial and final growth stages compared with control (Table 3). A similar trend for the tomato fresh leaf FRAP scavenging activity was also observed. In fact, we note a 6.18% increase in the scavenger effect for treatment T2 (18.68%) compared to treatment T3 (12.5%) in the initial growth stage and a slight decrease in T1 (12.28%), while in the final growth stage almost the same effect has been recorded between T1 and T3 (6368% and 63.68%, respectively).

Effect of NPK fertilizer on Antioxidant Enzyme Activities

The activities of the antioxidant enzymes catalase, ascorbate peroxidase (APX), and phenylalanine ammonia lyase (PAL) were not significantly different (Table 4).

Table 4. Effects of NPK fertilizer on the antioxidant enzymes of tomato plants

Enzymatic activities ($\mu M.min^{-1}. mg^{-1}$ of protein)	T1	T2	T3
CAT	0.15 ± 0.03^a	0.16 ± 0.04^a	0.12 ± 0.01^a
APX	13.89 ± 2.27^a	15.29 ± 3.36^a	11.21 ± 3.37^a
PAL	1.19 ± 0.08^a	1.21 ± 0.21^a	1.07 ± 0.12^a

Treatments: T1, 15 kg NPK ha^{-1}; T2, 20 kg NPK ha^{-1}; T3, 25 kg NPK ha^{-1}; CAT, catalase; APX, ascorbate peroxidase; PAL, phenylalanine ammonia lyase. Values followed by different small letters are significantly different at $p< 0.05$

Effect of NPK fertilizer on protein, total sugars, flavonoids, lycopene, and -carotene content

Our results indicate that NPK fertilization has no stimulatory effect on the accumulation of protein and total sugars in tomato fruits (Table 5). The unaffected contents of proteins and total sugar are in accordance with the results of chlorophyll pigment content. Likewise, the content of flavonoids, lycopene, and β-carotene in fruits of tomatoes treated with both rates of NPK fertilizer displays a similar trend of variation as that of total phenol content (Table 5).

Table 5. Effects of NPK fertilizer on total sugars, protein content, flavonoids, polyphenols, lycopene, and β-carotene contents of tomato fruits

Parameters	T1	T2	T3
Total sugars (mg/g FW)	3.76 ± 1.34^a	4.16 ± 0.25^a	5.43 ± 1.81^a
Proteins (mg/g FW)	0.165 ± 0.013^a	0.165 ± 0.024^a	0.171 ± 0.004^a
Flavonoid (µg QE/g FW)	0.062 ± 0.012^a	0.082 ± 0.020^a	0.088 ± 0.031^a
Polyphenols (mg GAE/g FW)	4.48 ± 0.42^a	4.85 ± 0.91^a	4.70 ± 0.88^a
Lycopene ($\mu g.g^{-1}$ FW)	58.43 ± 2.09^a	58.02 ± 2.13^a	58.62 ± 1.76^a
β-carotene ($\mu g.g^{-1}$ FW)	24.76 ± 4.19^a	25.09 ± 3.16^a	24.38 ± 3.02^a

Treatments: T1, 15 kg NPK ha^{-1}; T2, 20 kg NPK ha^{-1}; T3, 25 kg NPK ha^{-1}; µg QE/g FW, µg of quercetin equivalent per gram of fresh weight; mg GAE/g FW, mg of gallic acid equivalent per gram of fresh weight. Values followed by different small letters are significantly different at $p< 0.05$

Discussion

In the present study, application of NPK fertilizers influences morphological parameters of tomato growth: leaf area (cm^2), plant height (cm), tomato fruit number per plant, tomato weight per plant (kg), and total yield per hectare. At higher concentrations of NPK fertilizer (25 kg NPK ha^{-1}), all the growth parameters and yield are stimulated with no significant difference except for the number of fruits per plant and yield. This result was similar to the results of Zhang *et al*. (2020) on the positive response of Kiwi fruit (*Actinidia chinesis*) to mineral fertilization. Tomato yield was increased significantly by NPK. This can be expressed by mineral nutrition, and which considered the most important production factor for crop yield after water availability (Juárez-Maldonado *et al*., 2017).

Plant photosynthetic pigments such as chlorophyll and carotenoids play an essential role in controlling photosynthetic potential. Our results indicated an increase in average chlorophyll (b) and total chlorophyll content using the recommended dose of NPK fertilizer (T2: 20 kg NPK ha^{-1}), while the best content in chlorophyll a and carotenoids was observed using treatment T1 (15 kg NPK ha^{-1}) in the initial growth stage. However, in the final growth stage (fruit stage), the best amount of chlorophyll a and total chlorophyll was observed using the recommended dose of NPK fertilizer (T1). On the other hand, treatment T3 (25 kg ha^{-1}) showed the best result in chlorophyll b, while treatment T1 showed the optimum result in carotenoids. This may be explained by the fact that nitrogen participates directly in the formation of chlorophyll as well as the increase in the surface area of plant leaves. In previous studies, it was proven that nitrogen has several roles that can be mentioned in the photosynthetic pigments, the synthesis of the enzymes participating in carbon reduction, and the formation of the chloroplast. Our results are in agreement with those obtained by Pramanik and Bera (2013) in hybrid rice (*Oryza sativa* L.). Hokmalipour and Darbandi (2011) reported that nitrogen fertilizer has positive effects on the chlorophyll content of maize cultivars. Barzegar *et al*. (2020) reported that potassium and nitrogen fertilizer augmented chlorophyll content in sweet fennel (*Foeniculum vulgare* Mill.). Essential nutrients such as N, P and K play a major role in photosynthesis pigment biosynthesis, with N being a constituent of the porphyrin molecular structure of chlorophyll pigments (Barzegar *et al*., 2020). The positive effect of NPK fertilizer observed in the current study may be due to the fact that carbon reaction enzymes such as ribulose biphosphate carboxylase (RUBISCO) can be allocated more N (Akram, 2014). Phenolic compounds (total phenol and flavonoids) showed the best results using the medium treatment (the recommended dose of T2). The obtained results were in agreement with Biesiada *et al*. (2008), where was reported that the application of medium dose N fertilizer was the most suitable for lavender yielding, and that excess nitrogen fertilization decreased the concentration of phenolic compounds. Juan *et al*. (2008) also reported that increasing nitrogen supplies had a negative effect on total phenolic content. Other studies also showed the same effect on the phenolic content (Radi *et al*., 2003). Phenylalanine ammonia-lyase (PAL) is the rate-limiting enzyme of the phenylpropanoid pathway metabolism that catalyzes the deamination of L-phenylalanine to *trans*-cinnamic acid, and provides sufficient precursors for phenolic compound biosynthesis (Kováčik *et al*., 2007).

The application of NPK fertilizer to tomatoes did not have a significant effect on PAL activity in either of the NPK rates or growth phases. The results of our study indicate that a positive correlation exists between PAL enzyme activity and phenolic compounds (total phenolics and flavonoids). This may be one of the probable causes behind the unchangeable content of phenolic compounds, in addition to the bioavailability of essential nutrients like nitrogen, phosphorus, and potassium. However, when nitrogen availability is limited, plants enhance their production of carbon-based compounds, such as phenolic compounds (Verardo *et al*., 2013), due to stimulation of the enzymatic activities of phenol biosynthesis, such as PAL, or flavonoid biosynthesis, such as chalcone synthase (CHS) (Kováčik *et al*., 2007).

Proline content is usually indicated as a stress biomarker and explored in plant responses that can be accumulated under stress. According to previous studies done by Atanasova (2008), the high level of proline

accumulated by plants can be a sign of unbalanced nutrition. Other results indicate that proline may also play a role in regulating cytoplasmic pH, or as a carbon and nitrogen reserve that plants will use after a period of stress (Verslues and Sharma, 2010). Lipid peroxidation in tomato leaves, as indicated by the MDA contents, was not modified by NPK fertilizer. As an oxidative stress biomarker, the fact that the MDA amount is unchanged during treatment suggests that NPK fertilizer supply does not induce oxidative stress. This could explain why the levels of phenolic compounds and tocopherol in our sample remained constant. The steady DPPH and FRAP radical scavenging activity exhibited by the tomato plant might be due to the unchangeable contents of phenolic compounds found in the fresh leaf tomato extract. In previous studies, total phenolics and FRAP value were found to have a positive correlation, concluding that total phenolic compounds are responsible for antioxidant activity due to their redox properties, mainly the presence of the hydroxyl group, which can serve as electron donors (Amarowicz *et al*., 2004; Siddhuraju and Becker, 2007). Antioxidant enzymes play a key role in ROS (H_2O_2) scavenging and conversion into non-toxic water molecules. In the present study, application of NPK fertilizer to tomatoes did not have a statistically significant effect on APX and CAT activity in either of the NPK rates or growth stages. Under stress, potassium has a major role in the synthesis of proteins (Jones and Pollard, 1983). Reactive oxygen species are formed less frequently in stressed plants because of proteins like thioredoxin and glutaredoxin that help restore the reduced form of peroxiredoxin (Tripathi *et al*., 2009). Plants use the production of the antioxidant enzyme as a defense against oxidative stress. Ibrahim *et al*. (2012) reported that the increase of secondary metabolites could be explained by the increased CAT, APX, and PAL activities under high K fertilization and found a substantial link between total phenolics and flavonoids in the Kacip Fatimah (*Labisia pumilabenth*) plant.

In tomato fruit, β-carotene and lycopene are the most abundant carotenoids that play a key role in human health due to their antioxidant potential (Gerszberg *et al*., 2015). The amount of lycopene and carotenoids in NPK-treated tomatoes did not differ significantly in this investigation. The N application enhanced lycopene and total carotenoid content in tomatoes (Kuscu *et al*., 2014). Lycopene content is related to tomato type, growing system, and the ripening stage of the fruit in general.

Conclusions

The biochemical responses of plants to mineral fertilizer indicate that a medium dose is suitable, without overuse of fertilizers and pesticides, to reduce the negative impact on the agro-ecosystem.

Authors' Contributions

Conceptualization and Methodology: SN; Data curation, Funding acquisition and Investigation: ZB and HM; Project administration: MB; Resources: DDS; Writing - original draft: ZB and HM; Writing - review and editing: HM and SN. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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