SILICON TITANIUM OXIDE NANOPARTICLES CAN STIMULATE PLANT GROWTH AND THE PHOTOSYNTHETIC PIGMENTS ON LETTUCE CROP

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Our knowledge of the bioactivity of silicon titanium oxide nanoparticles (TiSiO₄ NPs) in crops is scarce, contrarily to TiO₂NPs and SiO₂NPs that are used in many industrial sectors, and have emerged in nanoagriculture (e.g., as pesticides or nanofertilisers). To evaluate the potential of using TiSiO₄ NPs in nanoagriculture, it is necessary to characterize their potential benefits on crops and the safety doses. Here, we report for the first time the bioactivity of TiSiO₄ NPs (up to 100 mg/L) in the model crop lettuce (*Lactuca sativa* L.) exposed for three weeks (from seeds/seedlings to pre-harvesting phase). The doses applied did not compromise the germination rate, and highly stimulated plant fresh matter. TiSiO₄ NPs had beneficial effects on photochemical processes by increasing chlorophyll levels. Effects on photosynthesis are less evident but TiSiO₄ NPs (100 mg/L) stimulated the photosynthetic potential, increasing F_v/F_m and ETR when compared to the 50 mg/L conditions. TiSiO₄ NPs did not influence the net photosynthetic rate and other Calvin-cycle variables. Soluble sugars and starch levels were overall maintained. In general, this first report on TiSiO₄ NPs bioactivity suggests that they did not have a toxic effect, and may be used to potentiate crops' growth. Principal component analysis (PCA) also shows that despite effects on photosynthetic performance is minimal regarding the control, the 50 and 100 mg/L doses strongly differ, with the lower dose promoting mostly pigment accumulation, while the higher dose slightly stimulates Photosystem II efficiency including the electron transport rate and other gas exchange parameters.

Key words: gas exchange, nanoagriculture, nanofertilisers, photosynthesis, TiSiO₄

Nanotechnologies are increasingly providing new and promising manufactured nanomaterials, including nanoparticles, to the agricultural sector (Parisi *et al.* 2015). The first data regarding the use of titanium in plant production was obtained at the beginning of the last century and has become an important field in plant nutrition (Kováčik *et al.* 2014). Silicon titanium oxide (TiSiO₄) is a ternary system

between titanium oxide (TiO₂) and silicon oxide (SiO₂) mixture that forms an orthorhombic structure, being increasingly used in multiple industries, particularly in the electronics sector (Garcia *et al.* 2009), ceramics (Varghese *et al.* 2011), optical instruments (Liu *et al.* 2017) and lubricant oils (Taheri *et al.* 2018). Despite their industrial potential, and unlike other metal nanoparticles, TiSiO₄ NPs remain

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unexplored in the nano-agroindustrial sector. Their antimicrobial activity was shown on *Vibrio fischeri* and *Salmonella typhimurium* (Pereira *et al.* 2011; Rodríguez-González *et al.* 2019). Also, using different concentrations up to 1,000 mg/kg soil or 1,000 mg/L (of aqueous suspensions), other authors as Bouguerra *et al.* (2016) observed that *E. andrei* only avoided the soils spiked with 1,000 mg/kg TiSiO₄ NPs. Despite still unknown, TiSiO₄ NPs may be safe at low doses to plants as no negative effects were found on corn and oat seedlings growth, and only small decreases of dry mass and fresh mass were observed on lettuce and tomato seedlings (Bouguerra *et al.* 2016). Some authors suggested that AgNPs induce more severe effects on the behavior of the terrestrial organisms than TiSiO₄ NPs, supporting the safe nature of these NPs. The lettuce market is huge being one of the most important leafy vegetable worldwide (Shatilov *et al.* 2019). Thus, is important to find strategies to improve the production of this crop.

On other hand, TiSiO₄ NPs are photocatalyst particles, and some NPs also with this characteristic, such as TiO₂, have been demonstrated at moderate doses to increase the photosynthetic performance, plant productivity, and quality (Shabbir *et al.* 2019; Gohari *et al.* 2020; Tighe-Neira *et al.* 2020). Also, their antimicrobial activity opens perspectives for crop protection as suggested by Rodríguez-González *et al.* (2019). Between the titanium oxide NPs (TiO₂ and TiSiO₄), TiO₂NPs are vastly more studied in terms of plant response, using physiological and biochemical approaches (El-Ramady *et al.* 2018; Ghosh *et al.* 2019; Silva *et al.* 2016; 2017a,b, 2019; Dias *et al.* 2019). Among the NPs with agricultural potential, those of TiO₂ are increasingly being used (Siddiqui *et al.* 2015; Duhan *et al.* 2017). The potential usefulness of TiO₂NPs in nanoagriculture may rely on their multiple functionalities, which include: a) antimicrobial activity (critical during germination and seedlings' first days) with the potential to manage plant diseases (Soni & Prakash 2012; Sekhon 2014; Duhan *et al.* 2017); b) stimulation of plant growth under certain doses (Jaberzadeh *et al.* 2013; Siddiqui *et al.* 2015). Likewise, SiO₂ (another component of the ternary system of TiSiO₄ NPs) has been reported to promote biofortification in plants, to be responsible for promoting the strengthening of

the cell walls (Guerreiro *et al.* 2016), has a mitigation role under abiotic stress (Behboudi *et al.* 2018), and is also increasingly being used on agricultural applications (Jampilek & Kráľová 2017; Luyckx *et al.* 2017).

As TiSiO₄ NPs impacts on plants remain unknown, we hypothesise that these NPs may combine the positive effects of the TiO₂ and SiO₂ NPs. Under moderate doses, TiO₂NPs were reported to stimulate plant biomass and positively regulate N metabolism (Yang *et al.* 2006). Recent data also pinpointed that TiO₂NPs were able to modulate plants' response to other stressors, lowering the Cu(II) and Cd phytotoxicity in rice (Wang *et al.* 2015; Ji *et al.* 2017) and increasing wheat tolerance to water deficit (Jaberzadeh *et al.* 2013). Germination may not be affected or even be stimulated by TiO₂NPs up to 750 mg/kg (Yang *et al.* 2015; Andersen *et al.* 2016; Maity *et al.* (2018). SiO₂NPs have been presented as a stimulator of yield and plant performance under unfavourable environment conditions, such as drought in the cotton plants (Behboudi *et al.* 2018).

Positive impacts in photosynthesis have been reported to TiO₂NPs and SiO₂NPs such as promoting electron transfer (Lei *et al.* 2007), increased transpiration rate (E), an increase of the net photosynthetic rate (Pn), stomatal conductance (gs) (Ashkavand *et al.* 2018). However, it should be noted that most studies on TiO₂NPs and SiO₂NPs bioactivity/phytotoxicity are performed during short periods. Studies using chronical exposure, whilst scarce, are much closer to real scenarios (Karunakaran *et al.* 2017; Silva *et al.* 2017a). Additionally, besides the species model, factors such as NPs size, shape, the presence and type of surface-coating, and NPs concentration vary greatly among studies, it can result in conflicting reports of their bioactivity (Cox *et al.* 2016; Silva *et al.* 2016; 2017b). Finally, phytotoxicity of high doses, particularly of the TiO₂NPs, is being unveiled by our and other groups, with description for example of the reduction of root growth, among other effects (Andersen *et al.* 2016; Cox *et al.* 2016; Karunakaran *et al.* 2017; Silva *et al.* 2016, 2017a,b, 2019).

Considering the complex data of the other NPs, it is critical to increase the available information on TiSiO₄ NPs bioactivity on plant growth and yield, to elucidate their potential for nanoagriculture. This in-

formation is crucial to evaluate if and how these NPs interact with crops' performance, their entrance into the food chain, and if they can replace/complement TiO₂NPs, SiO₂NPs, or both in nanoagroindustry.

Here, we hypothesise that TiSiO₄ NPs may retain some beneficial properties and effects of the TiO₂NPs and SiO₂NPs on crops, and thus may combine little or none toxicity with stimulation of growth and crop's performance, namely at the photosynthetic level. To test this hypothesis, lettuce plants (a major dicotyledonous model crop) were exposed to two different doses of TiSiO₄ NPs for three weeks, after which several photosynthesis-related endpoints (leaf gas exchange, chlorophyll *a* fluorescence, ribulose-1,5-bisphosphate carboxylase/oxygenase – RuBisCO amount, pigments and carbohydrates contents) were quantified.

MATERIAL AND METHODS

Nanoparticles stocks, characterization, and preparation of solutions

TiSiO₄ NPs (>99.8% purity) were purchased from Sigma-Aldrich (St. Louis, MO-USA) having, according to the supplier, a primary size <50nm. TiSiO₄ NPs dynamic light scattering (DLS) and zeta potential were characterized according to our current techniques (Bastos *et al.* 2017). A stock suspension of 1.0 g/L TiSiO₄ NPs (in distilled water) was used for the preparation of final nutrient solutions (Hoagland basal salt mixture, Sigma-Aldrich, USA) with concentrations up to 100 mg/L. All suspensions were sonicated for ≈20 min prior to use.

Seed germination and exposure to TiSiO₄ NPs

Seeds of *L. sativa* cv. 'Maravilha de Verão Canasta' (Vilmorin Jadin, France) were germinated in Petri dishes (100 seeds/petri dish), each with 10 mL of nutrient solution with different TiSiO₄ NPs concentrations: 0, 50, and 100 mg/L. Germination took place at 16 h light period (Photosynthetically active radiation – PAR ≈100 μmol/m²/s), 20±2°C, ≈50% relative humidity (%RH). One-week-old seedlings were hydroponically grown on the corresponding media at PAR ≈200 μmol/m²/s, 16 h light periods, 22±2°C, ≈45% RH. All media were renewed every 3 days, and pH was kept constant at ≈5.6. After 3

weeks, plant growth and biomass were determined by assessing shoot and root length, and fresh (FM) and dry (DM) matters. Morphological data were registered (senescence, chlorosis, necrotic spots, and abscission).

Photosynthetic analyses

L. sativa leaves in the rosette stage (first rosette leaves) grown at 0, 50, and 100 mg/L were used in photosynthetic assays. For pigments determination, leaves were homogenized in acetone: 50 mM Tris buffer (80:20) (pH ≈7.8) (Sims & Gamon 2002). Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), carotenoids, and anthocyanins were quantified at 470, 537, 647, and 663 nm.

The basal fluorescence yield of dark-adapted samples (F_0), the maximal fluorescence yield (F_m), and the variable fluorescence ($F_v = F_m - F_0$) were measured with the respective light conditions. The parameters F_0' , F_m' , and F_v' were also assessed in 30 min illuminated leaves. F_v/F_m and F_v'/F_m' , and the effective photochemical efficiency of PSII (Φ_{PSII}), photochemical quenching (q_p), and electron transport rate (ETR) were determined (Maxwell & Johnson 2000).

For leaf gas exchange analysis, the infra-red gas analyser (LCpro+, ADC, Hoddesdon, UK), operating in open mode, was used. Measurements took place under atmospheric CO₂ concentration with a saturating photosynthetic photon flux density of 600 μmol m²/s. Transpiration rate (E , mmol (H₂O) m²/s), stomatal conductance (g_s , mmol (H₂O) m²/s), net photosynthetic rate (P_n , μmol (CO₂) m²/s) and the ratio of intercellular CO₂ concentration to ambient CO₂ (C_i/C_a) were determined (Rodriguez *et al.* 2015).

Carbohydrates quantification

Total soluble sugars (TSS) and starch contents (mg/g FM) were quantified by the anthrone method (Osaki *et al.* 1991; Irigoyen *et al.* 1992).

RuBisCO quantification

Soluble proteins extracted from the first rosette leaves were quantified by the Bradford method (Sigma-Aldrich, USA). For RuBisCO analysis, 15 μg of protein were separated by SDS-PAGE, using protein molecular weight marker (Fermentas SM0441, ThermoFisher, USA) as a reference, and bands were

stained with 0.25% Coomassie Brilliant Blue R250 (Li *et al.* 2013). Relative RuBisCO quantification of large and small subunits was done after overnight incubation in 2 mL formamide at 50°C, reading absorbance at 595 nm. Results are expressed as $\text{ABS}_{\text{Rubisco content}} / \text{ABS}_{\text{Protein content}}$.

Statistical analysis

Experiments used 6–8 plant samples (treated individually or as leaf pools), and 3 independent technical replicates. Comparisons between the different conditions were made using the One-Way ANOVA test, using GraphPad Prism 6 (GraphPad Software, San Diego, California, USA). For different conditions, the Tukey Comparison Test ($p < 0.05$) was used. Multivariate analyses for data correlation used Principal component analysis (PCA) and were performed with CANOCO 5 (WUR, Netherland).

RESULTS

Nanoparticles characterization and effect on germination and plant growth

Taking into account the size of TiSiO_4 NPs (<50 nm in aqueous suspensions), these NPs tend to form

aggregates confirming previous data, and according to the DLS data, their hydrodynamic diameter was 2 to 3 times larger than the primary particle size, reaching average sizes of 115 ± 29 nm.

Seeds in all concentrations reached a germination rate of $\geq 99\%$, supporting no negative effects of these NPs on this crop's germination. Whilst root length and fresh matter (FM) of exposed plants were not affected compared to unexposed roots, both shoot length and, mainly shoots' FM, were in general stimulated by TiSiO_4 NPs compared to control plants (Table 1).

Photosynthetic pigments content and fluorescence

The average values of Chl *a* and *b* contents were stimulated by TiSiO_4 NPs (Figure 1a, b) with maximum values being achieved at 50 mg/L ($p < 0.05$). The ratio Chl *a*/Chl *b* was increased ($p < 0.05$) by both TiSiO_4 NPs doses (Figure 1c). Carotenoids' levels were not changed by TiSiO_4 NPs (Figure 1d). Finally, the anthocyanin levels were significantly increased at 50 mg/L TiSiO_4 NPs (Figure 1e).

Basal chlorophyll fluorescence of dark-adapted leaves (F_0) was slightly decreased ($p < 0.05$) by 100 mg/L TiSiO_4 NPs (Figure 2a). Maximum fluorescence (F_m) and variable fluorescence (Figure 2b, c)

Figure 1. Pigment contents (mg/kg (FM)) in plants exposed to TiSiO_4 NPs.

a) chlorophyll *a*; b) chlorophyll *b*; c) ratio Chl *a*/Chl *b*; d) Carotenoids; e) Anthocyanins; FM – Fresh Matter. All conditions were compared by Tukey multi comparison test using one-way ANOVA for $p < 0.05$.

were not compromised ($p > 0.05$) in exposed plants, but the increase of F_v/F_m ratio at 100 mg/L was significant compared to 50 mg/L condition ($p < 0.05$; Figure 2d). Regarding the light-adapted state, F'_v/F'_m ratio was affected increasing substantially at

100 mg/L compared to 50 mg/L (Figure 2f). For the photosystem efficiency, Φ_{PSII} was affected by $TiSiO_4$ NPs doses also with increases at 100 mg/L compared to 50 mg/L (Figure 2e). The q_p parameter was not affected by any NPs dose (Figure 2g). Lastly,

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Growth parameters of lettuce roots and shoots exposed to $TiSiO_4$ NPs

Plant area evaluated	Control	50 mg/L	100 mg/L
Root Length [cm]	12.83 ± 2.15	11.170 ± 1.56	12.46 ± 1.95
Shoot Length [cm]	9.94 ± 2.18	12.826 ± 2.30	10.44 ± 2.42
Root Fresh Matter [g]	8.49 ± 2.43	10.680 ± 3.52	10.62 ± 2.10
Shoot Fresh Matter [g]	11.22 ± 1.57	14.030 ± 1.33*	19.53 ± 2.54*

For the same condition and organ *means significantly different values ($p < 0.05$) in comparison with the control.

Figure 2. Fluorescence data (AU – arbitrary units) of plants exposed to $TiSiO_4$ NPs. a) F_0 – minimal fluorescence of dark-adapted leaves; b) F_v – variable fluorescence of dark-adapted leaves; c) F_m – maximum fluorescence of dark-adapted leaves; d) F_v/F_m – maximum quantum yield; e) Φ_{PSII} – effective quantum yield; f) F'_v/F'_m – maximum quantum yield in light-adapted condition; g) q_p – photochemical quenching; h) ETR – electron transport rate. All conditions were compared by Tukey multi comparison test using one-way ANOVA for $p < 0.05$.

ETR was stimulated ($p < 0.05$) by 100 mg/L dose compared to 50 mg/L (Figure 2h).

Gas exchange, RuBisCO, and carbohydrate levels

Leaf gas exchange parameters showed that the use of TiSiO₄ NPs did not affect the potential use

of CO₂ by *L. sativa* plants. Significant differences between each condition for all parameters (Figure 3a-e) were not found. Also, the parameters related to carbon assimilation, such as RuBisCO, TSS, and starch contents were not significantly affected by the TiSiO₄ NPs doses used (Figure 4a-c).

Figure 3. Leaf gas-exchange in plants exposed to TiSiO₄ NPs. a) E (mmol (H₂O) m²/s) – transpiration rate; b) gs – stomatal conductance; c) Pn (μmol (CO₂) m²/s) – net photosynthetic rate; d) Pn/gs ratio – Pn/gs – CO₂ assimilation efficiency; e) C_i/C_a – intercellular CO₂ ratio.

All conditions were compared by Tukey multi comparison test using one-way ANOVA for $p < 0.05$.

Figure 4. Relative RuBisCO content, starch, and total soluble sugars in plants exposed to TiSiO₄ NPs.

a) RuBisCO (ABS_{RC}/ABS_{TPC}); b) TSS (mg/g (FM)); c) Starch (mg/g (FM)); FM – Fresh Matter.

All conditions were compared by Tukey multi comparison test using one-way ANOVA for $p < 0.05$.

Figure 5. Principal component analysis (PCA) of functional responses of plants exposed to TiSiO_4 NPs

PCA (Figure 5) shows a distinct separation between all conditions used in physiological studies (0, 50, and 100 mg/L). PC1 (F1) explains 61.9% of the variance, while PC2 (F2) explains 39.1%. The control population is located on the down-right quadrant, and this parameter is the only located in the down-side of the Y-axis, associated with TSS and some fluorescence parameters (F_v and F_m). On the other hand, 50 mg/L is in the top-left quadrant and has positive scores for all photosynthetic pigments and g_s parameter. Regarding yield parameters of photosynthesis, such as Φ_{PSII} , F_v/F_m , F'_v/F'_m , ETR, P_n/g_s , RuBisCO, and starch contents, these are clusters on the top-right quadrant, corresponding to the 100 mg/L samples.

DISCUSSION

TiO_2 and SiO_2 NPs bioactivity in plants are currently under debate to support their potential use in nanoagriculture (Srivastava *et al.* 2015). Whilst most studies are focused on TiO_2 showing antibiotic activity and at some limited doses a stimulation of growth (but also toxicity at higher doses), SiO_2 is mostly pinpointed as a potential source of release of Si with demonstrated benefits to plant growth, and thus with potential as a nano fertiliser. The combination of Ti and Si may thus combine some of the

beneficial effects with minimal toxicity. However, the characterization of the TiSiO_4 NPs bioactivity in plants remains scarcely addressed. Moreover, most of the studies performed up to the moment with NPs used short term exposures, while agricultural purposes demand long term-exposure studies.

Ti is well known to be an element that when found in soils and waters used for plant growth, it enhances not only the plants' photosynthesis, but also their chlorophyll content, which promotes nutrient uptake, better stress tolerance, and improves crop yield and quality, making this a beneficial element (Lyu *et al.* 2017). On the other hand, the beneficial effects of silicon to plants are widely demonstrated (Murad *et al.* 2020) and its oxidised form (SiO_2 NPs) promoted plant protection against biotic and abiotic stress (Luyckx *et al.* 2017). Our work contains the first results addressing TiSiO_4 NPs photosynthetic bioactivity in plants, and we demonstrate that, after short-term exposure, moderate doses of TiSiO_4 NPs might stimulate lettuce growth, and may positively influence, in yet to unveil mechanisms, photosynthetic parameters, such as the increase of photosynthetic pigments at lower doses (50 mg/L) and gas exchange and PSII efficiency at higher doses (100 mg/L).

Recently we have demonstrated for *Triticum aestivum* that chronical exposure to TiO_2 NPs influences photosynthesis, and for example, 150 mg/L TiO_2 NPs had phytotoxic effects (stimulating oxidative stress and water disorders), which impaired plant growth, being toxicity more severe in shoots than in roots (Silva *et al.* 2017a; 2019). Using fresh and dry matter as growth indicators, and soils contaminated with extremely high doses (1,000 mg/kg soil_{DM}), Bouguerra *et al.* (2016) reported TiSiO_4 NPs phytotoxicity in lettuce and tomato plants. Comparing the available literature for TiO_2 NPs with our current data for TiSiO_4 NPs, there is evidence of lower toxicity of TiSiO_4 NPs, but further comparative studies under similar conditions must be conducted.

We demonstrate here that the interference of TiSiO_4 NPs with photosynthesis is complex and related to the dose, but overall a neutral or slight stimulation is found. Photophosphorylation is the source of ATP and $\text{NADPH} + \text{H}^+$ for the Calvin cycle to proceed, and its status here was evaluated according to changes in the chlorophyll quantity/ratio

for 50 mg/L TiSiO₄ NPs compared to control plants. It was evident that 50 mg/L TiSiO₄ NPs stimulated chlorophylls and anthocyanins contents (by increasing synthesis or preventing damage) which, besides photosynthesis, also have protective roles. The observed increase of Chl levels has been described in response to TiO₂ NPs, often paralleled with an increase of N uptake, but the targets of these NPs in the plant metabolism remain unknown (Lyu *et al.* 2017). Kheyrkhah *et al.* (2018) showed that SiO₂ and TiO₂ increased the chlorophylls and carotenoids content in oilseed rape cultivars. Also, Aliabadi *et al.* (2015) showed that 100 mg/L TiO₂ NPs increased the Chl *a* content and the ratio (Chl *a*/Chl *b*) in *T. aestivum*. However, the same study also showed that using 20 times more concentration (2,000 mg/L) reduced Chl content and its ratio. This study, in parallel with our data, demonstrates that moderate doses are capable to increase the photosynthetic potential, however, the species factor should be considered. These rises may increase the efficiency of CO₂ assimilation (Yang *et al.* 2006; Sanshez-Zabala *et al.* 2015; Lyu *et al.* 2017). Ti oxides NPs may also exert a protective role of photostability of chlorophylls, similarly to what was reported for silver nanoparticles (AgNPs) that slowed down the photodegradation of Chl *a* (Barazzouk *et al.* 2012). Thus, the increase of chlorophylls by TiSiO₄ NPs suggests either increased synthesis or an improved photostability of the pigments. This increase of pigments will improve the capacity for harvesting photons to foster the photophosphorylation process. The protective role of these pigments against photooxidation should also not be excluded. On the other hand, the absence of changes in fluorescence parameters and gas exchange compared to control were a positive indicator that the doses tested are not a toxic concentration of this compound for *L. sativa* growing in hydroponic systems, using the photosynthesis and carbon metabolism as control endpoints. Nevertheless, the use of TiO₂ NPs in another crop, such as *Solanum lycopersicum*, was capable to promote an increase in photosynthetic parameters (Qi *et al.* 2013). Besides, our data showed a particularity on the 100 mg/L conditions, as increases occurred in some photosynthetic parameters (F_v/F_m , Φ_{PSII} , F'_v/F'_m and ETR) compared to 50 mg/L. These results suggest that an increase of TiSiO₄ NPs relative to

50 mg/L can promote a positive effect in photosynthesis performance and the 100 mg/L concentration may not be the maximum endpoint of high photosynthetic performance for *L. sativa*.

Interestingly, the PCA analyses confirm a hormesis response in some parameters, shown by a negative correlation between 50 mg/L and the dark-adapted parameters of F_m , F_v , F_v/F_m , and F'_v/F'_m , while 100 mg/L had a similar negative correlation with F_0 . A higher F_0 can be interpreted as indicating irreversible damage to PSII (Bussotti *et al.* 2011), therefore the decrease of F_0 in exposed leaves may indicate a protective role exerted by these NPs on the PSII, decreasing the uncontrolled dissipation of heat. This hypothesis is also supported by the F_v/F_m ratio, which gives information on the maximum quantum efficiency of the PSII photochemistry. In TiSiO₄ NPs exposed plants, this ratio remains around the 0.79–0.84 values, which is an indicator of healthy plants (Maxwell & Johnson 2000) and indicates a balance between reduction of plastoquinone Q_A and its reoxidation by Q_B . This ratio is widely used as a sensitive endpoint for several stress-related photosynthetic disorders. For example, its disturbance was reported in some species exposed to TiO₂ NPs like *T. aestivum* (Dias *et al.* 2019). Also, in *Chlorella pyrenoidosa*, the inhibition of the photosynthetic activity was attributed to the damage of the reaction center of PSII (Middepogu *et al.* 2018).

Whilst the decrease of F_v/F_m may indicate some slowly relaxing quenching processes and photodamage to PSII reaction centres, reducing the maximum quantum efficiency of PSII photochemistry (Baker & Rosenqvist 2004), its increase – as observed in TiSiO₄ NPs exposed plants – suggests increased protection to chlorophyll photoinhibition. This protective role is also in accordance with the increase of chlorophyll levels in TiSiO₄ NPs exposed plants' leaves. Regarding light-adapted parameters, F'_v/F'_m was positively correlated with the 100 mg/L, meaning an increased capture efficiency of excitation energy by open PSII reaction centres in this NP concentration (Lima *et al.* 2002). Thus, we suggest that these NPs, directly or indirectly, stimulated the chlorophyll protection against photodamage (Wang *et al.* 2016), and/or increased chlorophyll synthesis to compensate for alleged increased damages as suggested by Middepogu *et al.* (2018). It should be

stressed/highlighted that this protective role may have slightly increased the efficiency of the photo-phosphorylation process, which may lead to an increase in the amount of ATP and NADPH available to be used in Calvin Cycle.

Data related to the gas exchange parameters did not show any responsive effect with the studied TiSiO_4 NPs doses to *L. sativa*. However, the multivariate analysis showed that g_s had a positive correlation with 50 mg/L and a Pn with opposite correlation for control plants and associated positively for 50 and 100 mg/L (Figure 5). A potential increase of Pn can be associated with the increase of CO_2 supply from outside to the intercellular airspaces of leaves (Dias *et al.* 2019). However, the non-significant changes in Pn do not follow the increase of Chl, nevertheless, the increase of Chl is not always accompanied by Pn increase (Da-yong *et al.* 2012). This is controlled by the stomatal function and the CO_2 diffusion from intercellular airspaces towards the carboxylation sites. These NPs do not interfere with the accumulation of CO_2 inside the mesophyll intercellular space, which suggests a proper function of RuBisCO and even a putative stimulation of its transcription, despite its relative amount not being changed. Different results with high concentrations of TiO_2 NPs were reported, namely the TiO_2 NPs concentration of 1,000 mg/L, which decreased CO_2 fixation, transpiration rate, and stomatal conductance (Da Costa & Sharma 2015). Also, we have recently demonstrated that TiO_2 NPs (anatase: rutile) are chronically applied to *T. aestivum*, decreased maximal and effective efficiency of PSII, net photosynthetic rate, transpiration rate, stomatal conductance, intercellular CO_2 concentration, and starch content (Dias *et al.* 2019). On the other hand, the application of SiO_2 NPs improves the gas exchange performance in *Passiflora edulis* with an increase of Pn, E, and g_s (Costa *et al.* 2018). Overall, our data suggest that low doses of TiSiO_4 NPs play some protective role in photosystem II, not having the negative impacts of TiO_2 NPs.

The protective role of TiSiO_4 NPs on the photosynthetic apparatus and the stimulation of RuBisCO subunits show stimulation of photosynthesis, supporting the observed plant growth increase. The TSS and starch levels are in line with all photosynthetic mechanisms under the effect of TiSiO_4 NPs.

However, TiO_2 NPs had a different effect on glycometabolism, as it decreased the levels of starch in *T. aestivum*, putatively as a mechanism to maintain the soluble sugars levels (Dias *et al.* 2019). Also, changes in carbohydrates after exposure to TiO_2 NPs were reported in cucumber fruits (Servin *et al.* 2013). Contrarily, despite negative effects on chlorophyll, TiO_2 NPs increased total sugar and reduced sugar in basil exposed to TiO_2 NPs (Tan *et al.* 2018). Those authors suggested that TiO_2 NPs led to the transfer of an active electron which might disrupt enzyme activities related to glycometabolism, like those as β -amylases (Thalmann & Santelia 2017), supporting the associated increase of sugar levels (Tan *et al.* 2018). Moreover, in TiSiO_4 NPs exposed plants, while starch content showed some PCA-correlation with Pn and RuBisCO content, it showed an opposite correlation to these parameters in control plants, supporting a trend to increase with these NPs (Figure 5), however, this concentration did not show significant differences. Our results demonstrate that TiSiO_4 NPs doses up to 100 mg/L did not affect the plant glycometabolism, while the positive correlation of some parameters such as Pn and RuBisCO for 100 mg/L showed that maybe the endpoints were not detected, and other upper doses could have a positive effect for *L. sativa*.

Mainly, some differences between the reported effects of TiO_2 and TiSiO_4 NPs perhaps occurred by the presence of Si element. Once this mineral is related with plant recovery for abiotic stress (Etesami & Jeong 2018) and is involved in photoprotection processes (Li *et al.* 2018), including the photosynthetic stimulation by the increase of the chlorophyll levels (Sarma *et al.* 2018), such as in *L. sativa* exposed to TiSiO_4 NPs.

CONCLUSIONS

In conclusion, this is one of the first approaches regarding the bioactivity of the understudied TiSiO_4 NPs, and it demonstrates that TiSiO_4 NPs stimulate plant growth, and different doses may promote photosynthetic apparatus and performance in different ways, namely lower doses may stimulate chlorophyll levels, while higher doses show a stimulatory trend to stimulate photosynthetic performance.

The tested doses did not have a negative effect on the net photosynthetic rate, nor influenced RuBisCO subunit amount. Being the first study on the biochemical effects of TiSiO_4 NPs this study also shows the need to better understand how manufactures nanoparticles interfere in crop performance and identify safety and realistic doses.

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Author contributions

C. Santos planned the experiments. N. Mariz-Ponte, S. Sario and R.J. Mendes performed all experimental assays and statistical analysis. J. Moutinho-Pereira and C.M. Correia performed IRGA/fluorimeter measurements. C. Correia supported N. Mariz-Ponte, S. Sario and R.J. Mendes in biochemical analyses. All authors contributed for statistical analysis, write and reviewed the manuscript.

Declaration of no conflict of interests

Authors state that they have no conflict of interests.

REFERENCES

- AL MURAD, M. – KHAN, A.L. – MUNEEB, S. 2020. Silicon in horticultural crops: Cross-talk, signalling, and tolerance mechanism under salinity stress. In *Plants*, vol. 9, no. 4, 460. DOI: 10.3390/plants9040460.
- ALIABADI, T. – AFSHAR, A.S. – NEMATPOUR, F.S. 2016. The effects of nano TiO_2 and Nano aluminium on the growth and some physiological parameters of the wheat (*Triticum aestivum*). In *Iranian Journal of Plant Physiology*, vol. 6, no. 2, pp. 1627–1635. DOI: 10.22034/ijpp.2016.539828.
- ANDERSEN, C. – KING, G. – PLOCHER, M. – STORM, M. – POKHREL, L. – JOHNSON, M. – RYGIEWICZ, P. 2016. Germination and early plant development of ten plant species exposed to titanium dioxide and cerium oxide nanoparticles. In *Environmental Toxicology and Chemistry*, vol. 35, no. 9, pp. 2223–9. DOI: 10.1002/etc.3374.
- ASHKAVAND, P. – ZARAFSHAR, M. – TABARI, M. – MIRZAIE, J. – NIKPOUR, A. – BORDBAR, S.K. – STRUVE, D. – GABRIEL, S.G. 2018. Application of SiO_2 nanoparticles as pretreatment alleviates the impact of drought on the physiological performance of *Prunus mahaleb* (rosaceae). In *Boletín De La Sociedad Argentina De Botanica*, vol. 53, no. 2, pp. 207–219. DOI: 10.31055/1851.2372.v53.n2.20578.
- BAKER, N. – ROSENQVIST, E. 2004. Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. In *Journal of Experimental Botany*, vol. 55, no. 403, pp. 1607–1621. DOI: 10.1093/jxb/erh196.
- BARAZZOUK, S. – BEKALÉ, L. – HOTCHANDANI, S. 2012. Enhanced photostability of chlorophyll-*a* using gold nanoparticles as an efficient photoprotector. In *Journal of Material Chemistry*, no. 22, pp. 25316–25324. DOI: 10.1039/C2JM33681B.
- BASTOS, V. – DE OLIVEIRA, J.F. – BROWN, D. – JONHSTON, H. – MALHEIRO, E. – DANIEL-DA-SILVA, A.L. – DUARTE, I.F. – SANTOS, C. – OLIVEIRA, H. 2016. The influence of Citrate or PEG coating on silver nanoparticle toxicity to a human keratinocyte cell line. In *Toxicology Letters*, vol. 249, pp. 29–41. DOI: 10.1016/j.toxlet.2016.03.005.
- BEHBOUDI, F. – SARVESTANI, Z.T. – KASSAEI, M.Z. – MODARES, S.A.M. 2018. Improving growth and yield of wheat under drought stress via application of SiO_2 nanoparticles. In *Journal of Agricultural Science and Technology*, vol. 20, no. 7, pp. 1479–1492.
- BUSSOTTI, F. – DESOTGIU, R. – CASCIO, C. – POLLASTRINI, M. – GRAVANO, E. – GEROSA, G. – MARZOU-LI, R. – NALI, C. – LORENZINI, G. – SALVATORI, E. – MANES, F. – SCHAUB, M. – STRASSER, R.J. 2011. Ozone stress in woody plants assessed with chlorophyll *a* fluorescence. A critical reassessment of existing data. In *Environmental and Experimental Botany*, vol. 73, pp. 19–30.
- BOUGUERRA, S. – GAVINA, A. – KSIBI, M. – RASTEIRO, M.D.A.G. – ROCHA-SANTOS, T. – PEREIRA, R. 2016. Ecotoxicity of titanium silicon oxide (TiSiO_4) nanomaterial for terrestrial plants and soil invertebrate species. In *Ecotoxicology and Environmental Safety*, vol. 129, pp. 291–301. DOI: 10.1016/j.ecoenv.2016.03.038.
- COSTA, B.N.S. – COSTA, I.D.J.S. – DIAS, G.D.M.G. – ASSIS, F.A.D. – PIO, L.A.S. – SOARES, J.D.R. – PASQUAL, M. 2018. Morpho-anatomical and physiological alterations of passion fruit fertilized with silicone. In *Pesquisa Agropecuária Brasileira*, vol. 53, no. 2, pp. 163–171. DOI: 10.1590/s0100-204x2018000200004.
- COX, A. – VENKATACHALAM, P. – SAHI, S. – SHARMA, N. 2016. Silver and titanium dioxide nanoparticle toxicity in plants: A review of current research. In *Plant Physiology and Biochemistry*, vol. 107, pp. 147–163. DOI: 10.1016/j.plaphy.2016.05.022.
- CUI, H. – ZHANG, P. – GU, W. – JIANG, J. 2009. Application of anatase TiO_2 sol derived from peroxotitanic acid in crop diseases control and growth regulation. In *NSTI-Nanotech*, vol. 2, pp. 286–289.
- DA COSTA, M.V.J. – SHARMA, P.K. 2015. Influence of Titanium dioxide nanoparticles on photosynthetic and biochemical processes in *Oryza sativa*. In *International Journal of Recent Scientific Research*, vol. 6, no. 1, pp. 2445–2451.
- DA-YONG, L. – ZHI-AN, Z. – DIAN-JUN, Z. – LI-YAN, J. – YUAN-LI, W. 2012. Comparison of net photosynthetic rate in leaves of soybean with different yield levels. In *Journal of Northeast Agricultural University* (English Edition), vol. 19, no. 3, pp. 14–19. DOI: 10.1016/S1006-8104(13)60017-3.
- DIAS, M.C. – SANTOS, C. – PINTO, G. – SILVA, A.M.S. – SILVA, S. 2019. Titanium dioxide nanoparticles impaired both photochemical and non-photochemical phases of photosynthesis in wheat. In *Protoplasma*, vol. 256, pp. 69–78. DOI: 10.1007/s00709-018-1281-6.

- DUHAN, J. – KUMAR, R. – KUMAR, N. – KAAUR, P. – NEHRA, K. – DUHAN, S. 2017. Nanotechnology: The new perspective in precision agriculture. In *Biotechnology Reports*, vol. 15, pp. 11–23. DOI: 10.1016/j.btre.2017.03.002.
- EL-RAMADY, H. – ABDALLA, N. – ALSHAAL, T. – EL-HENAWY, A. – ELMAHROUK, M. – BAYOUMI, Y. – SHALABY, T. – AMER, M. – SHEHATA, S. – FÁRI, M. – DOMOKOS-SZABOLCSY, É. 2018. Plant nano-nutrition: perspectives and challenges. In GOTHANDAM, K.M. et al. (Eds.), *Nanotechnology, Food Security and Water Treatment*. Springer International Publishing AG, Cham. pp. 129–161. DOI: 10.1007/978-3-319-70166-0.
- ETESAMI, H. – JEONG, B.R. 2018. Silicon (Si): Review and future prospects on the action mechanisms in alleviating biotic and abiotic stresses in plants. In *Ecotoxicology and Environmental Safety*, vol. 147, pp. 881–896. DOI: 10.1016/j.ecoenv.2017.09.063.
- FRAZIER, T. – BURKLEW, C. – ZHANG, B. 2014. Titanium dioxide nanoparticles affect the growth and microRNA expression of tobacco (*Nicotiana tabacum*). In *Functional & Integrative Genomics*, vol. 14, no. 1, pp. 75–83. DOI: 10.1007/s10142-013-0341-4.
- GHOSH, M. – GHOSH, I. – GODDERIS, L. – HOET, P. – MUKHERJEE, A. 2019. Genotoxicity of engineered nanoparticles in higher plants. In *Mutation Research/Genetic Toxicology and Environmental mutagenesis*, vol. 842, pp. 132–145. DOI: 10.1016/j.mrgentox.2019.01.002.
- GOHARI, G. – MOHAMMADI, A. – AKBARI, A. – PANAHIRAD, S. – DADPOUR, M.R. – FOTOPoulos, V. – KIMURA, S. 2020. Titanium dioxide nanoparticles (TiO₂ NPs) promote growth and ameliorate salinity stress effects on essential oil profile and biochemical attributes of *Dracocephalum moldavica*. In *Scientific Reports*, vol. 10, no. 1, pp. 1–14. DOI: 10.1038/s41598-020-57794-1.
- GUERRIERO, G. – HAUSMAN, J.F. – LEGAY, S. 2016. Silicon and the plant extracellular matrix. In *Frontiers in Plant Science*, vol. 7, pp. 463. DOI: 10.3389/fpls.2016.00463.
- GRACIA, L. – BELTRÁN, A. – ERRANDONEA, D. 2009. Characterization of the TiSiO₄ structure and its pressure-induced phase transformations: Density functional theory study. In *Physical Review B*, vol. 80, no. 9, 094105. DOI: 10.1103/PhysRevB.80.094105.
- IRIGOYEN, J.J. – EMERICH, D.W. – SÁNCHEZ-DÍAZ, M. 1992. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. In *Physiologia Plantarum*, vol. 84, no. 1, pp. 55–60. DOI: 10.1111/j.1399-3054.1992.tb08764.x.
- JABERZADEH, A. – MOAVENI, P. – MOGHADAM, H.R.T. – ZAHEDI, H. 2013. Influence of bulk and nanoparticles titanium foliar application on some agronomic traits, seed gluten and starch contents of wheat subjected to water deficit stress. In *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, vol. 41, no. 1, pp. 201–207. DOI: org/10.15835/nbha4119093.
- JAMPÍLEK, J. – KRÁĽOVÁ K. 2017. Nanomaterials for delivery of nutrients and growth-promoting compounds to plants. In PRASAD, R. – KUMAR M. – KUMAR V. (Eds.), *Nanotechnology*. Springer, Singapore. DOI: 10.1007/978-981-10-4573-8_9.
- Ji, Y. – ZHOU, Y. – MA, C. – FENG, Y. – HAO, Y. – RUI, Y. – WU, W. – GUI, X. – LE, V. – HAN, Y. – WANG, Y. – XING, B. – LIU, L. – CAO, W. – 2017. Jointed toxicity of TiO₂ NPs and Cd to rice seedlings: NPs alleviated Cd toxicity and Cd promoted NPs uptake. In *Plant Physiology and Biochemistry*, vol. 110, pp. 82–93. DOI: 10.1016/j.plaphy.2016.05.010.
- KOVÁČIK, P. – HAVRENTOVÁ, M. – ŠIMANSKÝ, V. 2014. Growth and yield stimulation of winter oilseed rape (*Brassica napus* L.) by Mg-Titanit fertiliser. In *Agriculture (Pol'nohospodárstvo)*, vol. 60, no. 4, pp.132–141. DOI: 10.1515/agri-2015-0002.
- KARUNAKARAN, G. – SURIYAPRABHA, R. – RAJENDRAN, V. – KANNAN, N. 2017. Influence of ZrO₂, SiO₂, Al₂O₃ and TiO₂ nanoparticles on maize seed germination under different growth conditions. In *IET Nanobiotechnology*, vol. 10, no. 4, pp. 171–177. DOI: 10.1049/iet-nbt.2015.0007.
- KHEYRKAH, M. – JANMOHAMMADI, M. – ABBASI, A. – SABAGHNIA, N. 2018. The effects of micronutrients (Fe And Zn) and beneficial nano-scaled elements (Si And Ti) on some morphophysiological characteristics of oilseed rape hybrids. In *Agriculture (Pol'nohospodárstvo)*, vol. 64, no. 3, pp. 116–127. DOI: 10.2478/agri-2018-0012.
- LEI, Z. – MINGYU, S. – CHAO, L. – LIANG, C. – HAO, H. – XIAO, W. – XIAOQING, L. – FAN, Y. – FENGQING, G. – FASHUI, H. 2007. Effects of Nanoanatase TiO₂ on photosynthesis of spinach chloroplasts under different light illumination. In *Biological Trace Element Research*, vol. 119, no. 1, pp. 68–76. DOI: 10.1007/s12011-007-0047-3.
- LI, D. – TIAN, M. – CAI, J. – JIANG, D. – CAO, W. – DAI, T. 2013. Effects of low nitrogen supply on relationships between photosynthesis and nitrogen status at different leaf position in wheat seedlings. In *Plant Growth Regulation*, vol. 70, no. 3, pp. 257–263. DOI: 10.1007/s10725-013-9797-4.
- LI, Z. – SONG, Z. – YAN, Z. – HAO, Q. – SONG, A. – LIU, L. – YANG, X. – XIA, S. – LIANG, Y. 2018. Silicon enhancement of estimated plant biomass carbon accumulation under abiotic and biotic stresses. A meta-analysis. In *Agronomy for Sustainable Development*, vol. 38, no. 3, article 26. DOI: 10.1007/s13593-018-0496-4.
- LIMA, A. – DAMATTA, F. – PINHEIRO, H. – TOTOLA, M. – LOUREIRO, M. 2002. Photochemical responses and oxidative stress in two clones of *Coffea canephora* under water deficit conditions. In *Environmental and Experimental Botany*, vol. 47, no. 3, pp. 239–247. DOI: 10.1016/S0098-8472(01)00130-7.
- LIU, H. – LIU, Z.T. – REN, J. – LIU, Q.J. 2017. Structural, electronic, mechanical, dielectric and optical properties of TiSiO₄: First-principles study. In *Solid State Communication*, vol. 251, pp. 43–49. DOI: 10.1016/j.ssc.2016.12.013.
- LUYCKX, M. – HAUSMAN, J.F. – LUTTS, S. – GUERRIERO, G. 2017. Silicon and plants: current knowledge and technological perspectives. In *Frontier in Plant Science*, vol. 8, 411. DOI: 10.3389/fpls.2017.00411.
- LYU, S. – WEI, X. – CHEN, J. – WANG, C. – WANG, X. – PAN, D. 2017. Titanium as a beneficial element for crop production. In *Frontier in Plant Science*, vol. 8, 597. DOI: 10.3389/fpls.2017.00597.
- MAITY, A. – NATARAJAN, N. – VIJAY, D. – SRINIVASAN, R. – PASTOR, M. – MALAVIYA, D.R. 2018. Influence of metal nanoparticles (NPs) on germination and yield of oat (*Avena sativa*) and berseem (*Trifolium alexandrinum*). In *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, vol. 88, pp. 595–607. DOI: 10.1007/s40011-016-0796-x.
- MAITY, A. – NATARAJAN, N. – PASTOR, M. – VIJAY, D. – GUPTA, C.K. – WASNIK, V.K. 2018. Nanoparticles influence seed germination traits and seed pathogen infection rate in forage sorghum (*Sorghum bicolor*) and cowpea (*Vigna unguiculata*). In *Indian Journal of Experimental Biology*, vol. 56, pp. 363–372.
- MAXWELL, K. – JOHNSON, G. 2000. Chlorophyll fluorescence – a practical guide. In *Journal of Experimental*

- Botany*, vol. 51, no. 345, pp. 659–668. DOI: 10.1093/jexbot/51.345.659.
- MEENA, R. – RUCHITA, P. – NARAYAN, S. – MADHU, R. – PAULRAJ, R. 2012. Comparative study of TiO₂ and Ti-SiO₄ nanoparticles induced oxidative stress and apoptosis of HEK-293 cells. In *Advanced Materials Letters*, vol. 3, no. 6, pp. 459–465. DOI: 10.5185/amlett.2012.icnano.157.
- MIDDEPOGU, A. – HOU, J. – GAO, X. – LIN, D. 2018. Effect and mechanism of TiO₂ nanoparticles on the photosynthesis of *Chlorella pyrenoidosa*. In *Ecotoxicology and Environmental safety*, vol. 161, pp. 497–506.
- OSAKI, M. – SHINANO, T. – TADANO, T. 1991. Redistribution of carbon and nitrogen-compounds from the shoot to the harvesting organs during maturation in field crops. In *Soil Science and Plant Nutrition*, vol. 37, no. 1, pp. 117–128. DOI: 10.1080/00380768.1991.10415017.
- PARISI, C. – VIGANI, M. – RODRÍGUEZ-CEREZO, E. 2015. Agricultural nanotechnologies: What are the current possibilities? In *Nanotoday*, vol. 10, no. 2, pp. 124–127. DOI: 10.1016/j.nantod.2014.09.009.
- PEREIRA, R. – ROCHA-SANTOS, T.A.P. – ANTUNES, F.E. – RASTEIRO, M.G. – RIBEIRO, R. – GONÇALVES, F. – SOARES, A.M.V.M. – LOPES, I. 2011. Screening evaluation of the ecotoxicity and genotoxicity of soils contaminated with organic and inorganic nanoparticles: the role of ageing. In *Journal of Hazardous Material*, vol. 194, pp. 345–354. DOI: 10.1016/j.jhazmat.2011.07.112.
- QI, M. – LIU, Y. – LI, T. 2013. Nano-TiO₂ improve the photosynthesis of tomato leaves under mild heat stress. In *Biological Trace Element Research*, vol. 156, no. 1–3, pp. 323–328. DOI: 10.1007/s12011-013-9833-2.
- RODRÍGUEZ, E. – SANTOS, C. – AZEVEDO, R. – CORREIA, C. – MOUTINHO-PEREIRA, J. – FERREIRA DE OLIVEIRA, J.M. – DIAS, M.C. 2015. Photosynthesis light-independent reactions are sensitive biomarkers to monitor lead phytotoxicity in a Pb-tolerant *Pisum sativum* cultivar. In *Environmental Science and Pollution Research*, vol. 22, no. 1, pp. 574–585. DOI: 10.1007/s11356-014-3375-9.
- RODRÍGUEZ-GONZÁLEZ, V. – TERASHIMA, C. – FUJISHIMA, A. 2019. Applications of photocatalytic titanium dioxide-based nanomaterials in sustainable agriculture. In *Journal of Photochemistry and Photobiology C: Photochemistry Reviews*, vol. 40, pp. 49–67. DOI: 10.1016/j.jphotochemrev.2019.06.001.
- SANCHEZ-ZABALA, J. – GONZÁLEZ-MURUA, C. – MARINO, D. 2015. Mild ammonium stress increases chlorophyll content in *Arabidopsis thaliana*. In *Plant Signaling & Behaviour*, vol. 10, no. 3, e991596. DOI: 10.4161/15592324.2014.991596.
- SARMA, R.S. – SHANKHDHAR, D. – SRIVASTAVA, P. – SHANKHDHAR, S.C. 2018. Influence of silicon solubilizers on Silicon content, chlorophyll content (mg.g⁻¹) and photosynthetic efficiency in leaves at three different growth stages in rice genotypes. In *Journal of Pharmacognosy and Phytochemistry*, vol. 7, no. 2, pp. 2552–2558.
- SERVIN, A.D. – MORALES, M.I. – CASTILLO-MICHEL, H. – HERNÁNDEZ-VIEZCAS, J.A. – MUNOZ, B. – ZHAO, L. – NÚÑEZ, J.E. – PERALTA-VIDEA, J.R. – GARDEA-TORRESDEY, J.L. 2013. Synchrotron verification of TiO₂ accumulation in cucumber fruit: a possible pathway of TiO₂ nanoparticle transfer from soil into the food chain. In *Environmental Science & Technology*, vol. 47, no. 20, pp. 11592–11598. DOI: 10.1021/es403368j.
- SEKHON, B.S. 2014. Nanotechnology in agri-food production: an overview. In *Nanotechnology, Science and Application*, vol. 7, pp. 31–53. DOI: 10.2147/NSA.S39406.
- SHABBIR, A. – KHAN, M.M.A. – AHMAD, B. – SADIQ, Y. – JALEEL, H. – UDDIN, M. 2019. Efficacy of TiO₂ nanoparticles in enhancing the photosynthesis, essential oil and khusimol biosynthesis in *Vetiveria zizanioides* L. Nash. In *Photosynthetica*, vol. 57, no. 2, pp. 599–606. DOI: 10.32615/ps.2019.071.
- SHATILOV, M.V. – RAZIN, A.F. – IVANOVA, M.I. 2019. Analysis of the world lettuce market. In *IOP Conference Series: Earth and Environmental Science*, vol. 395, no. 1, p. 012053. IOP Publishing. DOI: 10.1088/1755-1315/395/1/012053.
- SIDDIQUI, M.H. – AL-WHAIBI, M.H. – FIROZ, M. – AL-KHAISHANY, M. 2015. Role of nanoparticles in plants. In SIDDIQUI, M.H. et al. (Eds.), *Nanotechnology and Plant Sciences*, Chapter 2, pp. 19–35. DOI: 10.1007/978-3-319-14502-0_2.
- SILVA, S. – OLIVEIRA, H. – CRAVEIRO, S.C. – CALADO, A.J. – SANTOS, C. 2016. Pure anatase and rutile + anatase nanoparticles differently affect wheat seedlings. In *Chemosphere*, vol. 151, pp. 68–75. DOI: 10.1016/j.chemosphere.2016.02.047.
- SILVA, S. – CRAVEIRO, C. – OLIVEIRA, H. – SILVA, A.M.S. – SANTOS, C. 2017a. Wheat chronic exposure to TiO₂-nanoparticles: Cyto- and genotoxic approach. In *Plant Physiology and Biochemistry*, vol. 121, pp. 89–98. DOI: 10.1016/j.plaphy.2017.10.013.
- SILVA, S. – OLIVEIRA, H. – SILVA, A.M.S. – SANTOS, C. 2017b. The cytotoxic targets of anatase or rutile + anatase nanoparticles depend on the plant species. In *Biologia Plantarum*, vol. 61, no. 4, pp. 717–725. DOI: 10.1007/s10535-017-0733-8.
- SILVA, S. – DE OLIVEIRA, J.M.P.F. – DIAS, M.C. – SILVA, A.M. – SANTOS, C. 2019. Antioxidant mechanisms to counteract TiO₂-nanoparticles toxicity in wheat leaves and roots are organ dependent. In *Journal of Hazardous Materials*, vol. 380, 120889. DOI: 10.1016/j.jhazmat.2019.120889.
- SIMS, D.A. – GAMON, J.A. 2002. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. In *Remote Sensing of Environment*, vol. 81, no. 1–2, pp. 337–354. DOI: 10.1016/S0034-4257(02)00010-X.
- SONI, N. – PRAKASH, S. 2012. Efficacy of fungus mediated silver and gold nanoparticles against *Aedes aegypti* larvae. In *Parasitology Research*, vol. 110, no. 1, pp. 175–184. DOI: 10.1007/s00436-011-2467-4.
- SRIVASTAVA, V. – GUSAIN, D. – SHARMA, Y. 2015. Critical review on the toxicity of some widely used engineered nanoparticles. In *Industrial & Engineering Chemistry Research*, vol. 54, no. 24, pp. 6209–6233. DOI: 10.1021/acs.iecr.5b01610.
- TAHERI, R. – KOSASIH, B. – ZHU, H. – TIEU, A.K. 2018. Dual effects of TiSiO₄ composite nanoparticles on dispersion stability and lubrication performance of vegetable oil-in-water emulsions. In *Lubrication Science*, vol. 31, no. 1–2, DOI: 10.1002/lis.1443.
- TAN, W. – DU, W. – DARROUZET-NARDI, A.J. – HERNÁNDEZ-VIEZCAS, J.A. – YE, Y. – PERALTA-VIDEA, J.R. – GARDEA-TORRESDEY, J.L. 2018. Effects of the exposure of TiO₂ nanoparticles on basil (*Ocimum basilicum*) for two generations. In *Science of Total Environment*, vol. 636, pp. 240–248. DOI: 10.1016/j.scitotenv.2018.04.263.
- THALMANN, M. – SANTELLA, D. 2017. Starch as a determinant of plant fitness under abiotic stress. In *New Phytologist*, vol. 214, no. 3, pp. 943–951. DOI: 10.1111/nph.14491.
- TIGHE-NEIRA, R. – REYES-DÍAZ, M. – NÚÑES-NESE, A. – RECIO, G. – CARMONA, E. – CORGNE, A. – RENGEL, Z. – INOSTROZA-BLANCHETEAU, C. 2020. Titanium di-

- oxide nanoparticles provoke transient increase in photosynthetic performance and differential response in antioxidant system in *Raphanus sativus* L. In *Scientia Horticulturae*, vol. 269, 109418. DOI: 10.1016/j.scienta.2020.109418.
- ZIDAR, P. – KOS, M. – ILIC, E. – MAROLT, G. – DROBNE, D. – JEMEC KOKALJ, A. 2019 Avoidance behaviour of isopods (*Porcellio scaber*) exposed to food or soil contaminated with Ag- and CeO₂ – nanoparticles. In *Applied Soil Ecology*, vol. 141, pp. 69–78.
- VARGHESE, J. – JOSEPH, T. – SEBASTIAN, M.T. 2011. Sol-gel derived TiSiO₄ ceramics for high-k gate dielectric applications. In *AIP Conference Proceedings*, vol. 1372, no. 1, pp. 193–197. DOI: 10.1063/1.3644442.
- WANG, Y. – PENG, C. – FANG, H. – SUN, L. – ZHANG, H. – FENG, J. – DUAN, D. – LIU, T. – SHI, J. 2015. Mitigation of Cu(II) phytotoxicity to rice (*Oryza sativa* L.) in the presence of TiO₂ and CeO₂ nanoparticles combined with humic acid. In *Environmental Toxicology and Chemistry*, vol. 34, no. 7, pp. 1588–1596. DOI: 10.1002/etc.2953.
- WANG, X.P. – YANG, X.Y. – CHEN, S.Y. – LI, Q.Q. – WANG, W. – HOU, C.J. – GAO, X. – WANG, L. – WANG, S.C. 2016. Zinc oxide nanoparticles affect biomass accumulation and photosynthesis in *Arabidopsis*. In *Frontier in Plant Science*, vol. 7, no. 6, 1243. DOI: 10.3389/fpls.2015.01243.
- YANG, Z. – CHEN, J. – DOU, R. – GAO, X. – MAO, C. – WANG, L. 2015. Assessment of the phytotoxicity of metal oxide nanoparticles on two crop plants, maize (*Zea mays* L.) and rice (*Oryza sativa* L.). In *International Journal of Environmental Research and Public Health*, vol. 12, no. 12, 15100–9. DOI: 10.3390/ijerph121214963.
- YANG, F. – HONG, F.S. – YOU, W.J. – LIU, C. – GAO, F.Q. – WU, C. – YANG, P. 2006. Influences of nano-anatase TiO₂ on the nitrogen metabolism of growing spinach. In *Biological Trace Element Research*, vol. 110, no. 2, pp. 179–190. DOI: org/10.1385/BTER:110:2:179.
- YAO, K.S. – WANG, D.Y. – HO, W.Y. – YAN, J.J. – TZENG, K.C. 2007. Photocatalytic bactericidal effect of TiO₂ thin film on plant pathogens. In *Surface and Coatings Technology*, vol. 201, no. 15, pp. 6886–6888. DOI: 10.1016/j.surfcoat.2006.09.068.
- ZAHRA, Z. – ARSHAD, M. – RAFIQUE, R. – MAHMOOD, A. – HABIB, A. – QAZI, I.A. – KHAN, S.A. 2015. Metallic nanoparticle (TiO₂ and Fe₃O₄) application modifies rhizosphere phosphorus availability and uptake by *Lactuca sativa*. In *Journal of Agricultural and Food Chemistry*, vol. 63, no. 31, pp. 6876–6882. DOI: 10.1021/acs.jafc.5b01611.

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