Foliar application of titanium on potato crop

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Abstract

Although titanium (Ti) is not considered a nutrient, researches demonstrate that Ti leaf application can provide beneficial effects on plants growth, according to the positive stimulation of plant metabolism. The objective of this study was to evaluate the influence of foliar applications of Ti levels on the metabolism, nutrient uptake and yield of potato crop. The experiment was conducted in a randomized complete block design, being composed of Ti levels (0, 10.2, 15.3, 20.4 and 22.9 g Ti ha^{-1}), divided into three applications during the growth stage, tuberization stage and tuber filling stage. Foliar applications of Ti increase the chlorophyll content (Spad value) in the tuber filling stage. Increasing Ti levels linearly reduce the Mn, lipid peroxidation (PL) and urease content and increase the activity of peroxidase (POD), nitrate reductase (ANR), catalase (CAT), proline and Fe content in the growth stage. In the tuberization stage, at high levels of Ti, there is an increase in urease activity and CAT while ANR has its lowest value. In the tuber filling stage, high Ti levels are related to high Fe, Mn, high ANR and superoxide dismutase (SOD) activity and low PL activity. The ideal level of titanium applied by leaf for greater yield of tubers is 11.3 g of Ti ha^{-1}. Ti positively affects potato development, so the nutrient has the potential to be used to improve production, being another alternative to add to the sustainability of crops.

Keywords: beneficial element, enzymatic activity, nutrient absorption, *Solanum tuberosum* L.

Introduction

Potato (*Solanum tuberosum*) has a good taste, contain important mineral compounds and essential vitamins and presents high yield (Nurmanov et al., 2019). Potato is the largest non-cereal food crop worldwide, considering the nutritional value the utilization of potato is expected to be improved (Zhang et al., 2017), especially in developing countries (Wijesinha-Bettoni & Mouillé, B. 2019).

The culture is highly responsive and demanding for fertilization and receives high levels of macronutrients in order to guarantee high productivity (Fernandes et al., 2015). The efficient use of nutrients by the crop calls attention to the response of other elements such as micronutrients or beneficial elements.

Titanium (Ti) can help increase the photosynthetic rate, stimulate enzymatic activities, participate in proteins and chlorophyll synthesis and contribute to the control of plant diseases (Qureshi et al., 2018). Kleiber & Markiewicz (2013) reported benefits of applying Ti to tomatoes, with an increase in biomass, chlorophyll, contents of nitrogen, phosphorus, calcium and magnesium and yields improvement. Shabbir et al. (2019) and Ahmad et al. (2018) found photosynthesis and essential oil yield improvements in *Mentha piperita* and *Vetiveria zizanioides*, respectively.

Despite the benefits reported in the literature, there is no information on levels, formulations, methods, application periods and crop responses to fertilizers containing Ti (Bacilieri et al., 2017). Research on the effects of Ti is growing and efforts to understand its effects on plants are expanding because there is currently a dual nature between beneficial and toxic effects depending on many experimental factors (Mukherje et al., 2016; Cox et al., 2017).

There is much to be discovered about Ti processes

and interferences in plants, disease suppression and crop yield increase (Zuverza-Mena et al., 2017). Studies on the possible influence of Ti on potato crop as well as understanding of its effects on metabolism through the activity of enzymes or the absorption of some nutrients already considered essential are important to optimize the resources used for production, reduce costs, and promote increased in productivity.

The objective of this study was to evaluate the influence of foliar Ti levels on the metabolism, nutrient uptake, and productivity of the potato crop, Agate cultivar.

Material and methods

The experiment was conducted in Uberlandia-MG (Fisioplant), in an experimental area, from August to November of 2014. The climate of the area is Aw (tropical seasonal of Savanna) in Koppen classification. The average annual rainfall and temperature are around 1200 mm year^{-1} and 25°C, with the rainfall concentrated between the months of November and March. Relative humidity ranges from 50-60% to 85-90% during the rainy season. The soil of the experimental area is classified as Red Latosol and medium texture. The chemical attributes are shown in Table 1.

Table 1. Chemical attributes of the soil in the 0.00 - 0.2 m at the Experimental Station.

pH H$_2$O	P	K	Al^{3+}	Ca^{2+}	Mg^{2+}	H+Al	SB	t	T	V	O.M.
	mg dm^{-3}					Cmolc dm^{-3}					%
5.5	24.4	200	0.1	2.1	0.5	3.8	3.11	2.17	6.91	45	4.2

Soil preparation was carried out in a conventional manner with the following sequence of operations: heavy weeding, scarification and light harrowing on the eve of planting.

Planting grooves were mechanically opened, with furrow-planter spacing of 0.75 m between rows. The formulated fertilizer 08-28-16 was dispensed at the dosage of 350 kg ha^{-1} manually. Subsequently the fertilizer was incorporated into the soil with a hoe, and the seed tubers (type III) were distributed manually, at a spacing of 0.20 m. At 30 days after emergence (DAE), the ridging and fertilizer application of 150 kg ha^{-1} of 20-05-20 were made.

In the experiment area, there was water supplementation through sprinkler irrigation. The plants received the amount of water required for full development throughout the growing period (between 450 and 550 mm). The experimental plots consisted of six lines of four meters in length each and spacing was 0.75 m between lines, the size of the plots was 18.0 m².

The treatments consisted of five levels of Ti (0, 10.2, 15.3, 20.4 and 22.9 g Ti ha^{-1}) divided into three applications, one third applied in the growth stage, one third in the tuberization stage and one third in the tuber filling stage. A fertilizer composed of 8.5 g L^{-1} of soluble Ti, 50.0 g L^{-1} of soluble MgO and 10 g of soluble SO$_3$ was used as the source of Ti. The applications were started when plants presented 3-6 leaves, stage 13-16 according to the BBCH scale (Meier, 2001) with interval of 15 days for the second and 13 days for the third application. Thus, the applications were performed at 23, 36 and 50 DAE.

A CO$_2$ pressurized spool was used with a constant pressure of 60 lbf.pol^{-2}, equipped with a bar with three spray tips type 110.02, regulated for a flow with the volume of syrup equivalent to 200 L ha^{-1}.

The amount of leaf chlorophyll was determined with a Soil Plant Analysis Development, (SPAD, brand SPAD-502) chlorophyll meter. The leaves selected were in perfect condition, free of attack of fully-expanded pests or diseases. The measurement occurred at 10 days after the third application of the treatments.

To evaluate leaf enzyme activity and nutrient content, a fully expanded leaf was collected in the middle third of five plants according to the methodology proposed by Malavolta et al. (1997).

The activity of the enzyme nitrate reductase (ANR - EC 1.6.6.1) was evaluated following the methodology proposed by Cataldo (1975). Urea activity (E.C 3.5.1.5) was determined by adapting the methods proposed by Hogan, Swift and Done (1983). Proline determination was performed by the method described by Bates et al. (1973), and SOD activity was determined as proposed by Giannopolitis & Ries (1977).

The activity of the catalase enzyme (EC 1.11.1.6) was measured by a spectrophotometer at 240 nm, by monitoring hydrogen peroxide variation absorption as proposed by Peixoto et al. (1999). The activity of POD (POD - EC 1.11.1.7), also called pyrogallolperoxidase, was determined according to the methodology proposed by Teisseire & Guy (2000). Lipid peroxidation was determined according to the technique of Heath & Packer (1968).

The results were expressed as nmol of substance reactive to thiobarbituric acid (TBARS) per gram of

fresh matter. The nutrient content of nitrogen, iron, copper, manganese and zinc in leaves and tubers were determined according to the methodology proposed by EMBRAPA (2009).

The harvested tubers were washed with a millimeter ruler. The transverse diameter was measured and the tubers were weighed for commercial yield and the mean mass of tubers was determined.

The results were submitted for variance analysis; the effects of Ti levels were evaluated by regression analysis at each stage (plant growth, tuberization and tuber filling).

Results and Discussion

The SPAD value did not differ between Ti levels in the stages of growth and tuberization. At 10 DAA the maximum estimated level of Ti (19.56 g ha^{-1}) occurred at a SPAD value of 38.68 (Figure 1).

$$Y = -0.00003x^2 + 1.19x + 27.01$$
$$R^2 = 93.12\%$$
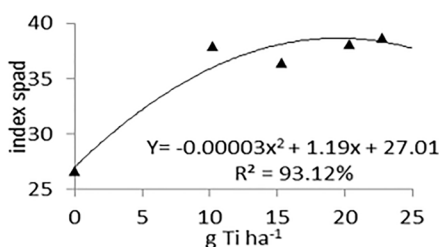
Figure 1. SPAD index in potato leaves in tuber filling stage as a function of Ti levels.

The SPAD can provide a fast, convenient and reliable method for farmers and agronomic consultants to diagnose the N status of the crop (Padilla et al., 2017). When the potato plant is in the advanced stage of development, the leaves begin to gradually have a reduction in the chlorophyll content and show a sharp green coloration. In this way, Ti applications can contribute to higher levels of chlorophyll thus avoiding early senescence of the plants. Researches demonstrate increases in the SPAD index around 13 to 17% with an increase in Ti application from 3.4 to 6.8 g ha^{-1} (Radkowski et al., 2015).
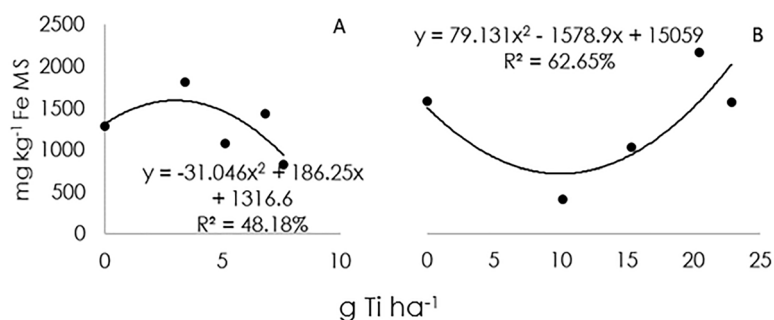
Nutrients absorption

The Ti levels did not influence N levels in the evaluated stages, which ranged from 40.6 to 42.1; 36.5 to 40.37 and 21.72 to 31.63 mg kg^{-1} DM in tuber growth, tuberization and tuber filling stages, respectively.

The phosphate and ammonia in plant cell walls may contribute to the association between Ti and plants, in part promoting toxic effects at high levels (Pittol et al., 2017). This fact was not observed in the present work due to low contents found during the potato growth.

Haghighi et al. (2012), working with tomato, verified that 1 mg^{-1} of Ti in hydroponic nutrient solution made it possible to reduce the N supply by up to 50% without affecting the dry and fresh weight of the shoot and root efficiency and thus increases the N use by plants.

In all stages, Cu levels in the leaves did not show significant effects as a function of Ti levels. Cu contents ranged from 5 to 6.69; 137.1 to 217.07 and 25.98 to 42.04 mg kg^{-1} in the tuber growth, tuberization and tuber filling stages, respectively. The values were lower than the ideals proposed by Fernandes et al. (2011) (41 mg kg^{-1} DM), only in the plant growth stage.

The high content in the middle stage of potato development is possibly related to applications of cupric pesticides, since at that stage the plants are more susceptible to biotic disturbances. The content 4 times higher than found by Fernandes et al. (2011) in the same period may be related to time of application, which was near the date of collection of the leaf samples, with subsequent stabilization in the range found by Fernandes et al. (2011).

Fe supports the application of Ti up to 3 g of Ti ha^{-1} in growth stage, with a maximum content of 1595.94 mg kg^{-1} DM. In the tuberization stage, Ti does not interfere in Fe accumulation, with variation between 1037.84 to 1654.24 mg kg^{-1} DM, and in the tuber filling stage, levels higher than 9.98 mg kg^{-1} DM promoted an increase in Fe accumulation (Figure 2).

A: $$y = -31.046x^2 + 186.25x + 1316.6$$
$$R^2 = 48.18\%$$

B: $$y = 79.131x^2 - 1578.9x + 15059$$
$$R^2 = 62.65\%$$

Figure 2. Fe content (mg kg^{-1} DM) in potato leaves as a function of Ti levels, in stages of plant growth (A) and tuber filling (B).

The initial Fe content reduction in the leaves can be attributed to the competitive inhibition between Fe x Ti because they are ions absorbed in the bivalent form. Kuzel et al. (2003) attributed the increase in nutrient level after the application of Ti to a mechanism called "hormesis"; this theory show how a substance when applied at low doses has the capacity to cause a physiological stimulus in the plant and the nutrient absorption increases.

On the other hand, Ti has a synergistic action with Fe, and when the plants experience Fe deficiency, Ti can help to induce the expression of genes related to Fe acquisition. This increases the uptake and utilization of Fe and, subsequently, improves plant growth (Lyu et al., 2017).

The higher Fe content in the leaves may be advantageous because the tuber filling stage is the period of higher demand for Fe in the Agate potato representing up to 81% of the accumulated Fe (Fernandes, 2011).

The Mn content decreases with increasing Ti doses in the growing stage. At the highest level, there was a 23% reduction in Mn content (48.89 mg kg^{-1} DM) (Figure 3). This result indicates that may there was a competitive inhibition between Ti x Mn or with higher plant growth there may have been dilution of this nutrient in the leaves.

$$y = -1.926x + 63.535 \quad R^2 = 81.88\%$$
$$y = 0.1809x^2 - 2.6422x + 44.806 \quad R^2 = 66.24\%$$
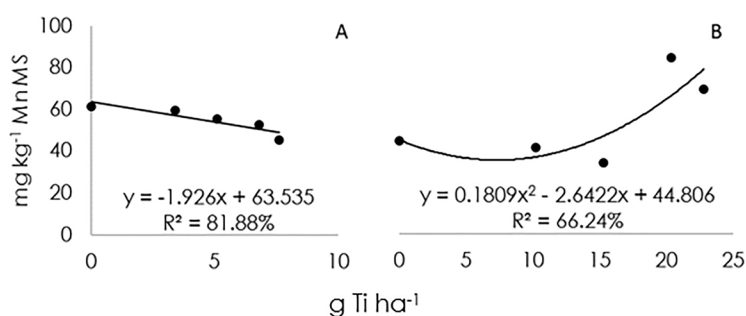
Figure 3. Mn content (mg kg^{-1} DM) in potato leaves as a function of Ti levels, in stages of plant growth (A) and tuber filling (B).

These results corroborate with those obtained by Malinowsk & Kalembas (2012) that after two foliar applications of 0.0001% and 3.6% of Tytanit fertilizer there was found a reduction of Mn levels in celery leaves (*Apium graveolens* L.). In addition, Kuzel et al. (2003) observed that foliar application of 50 mg ha^{-1} of Ti in the growth stage reduced Mn content in dry mass of oat leaves by 1.52 times.
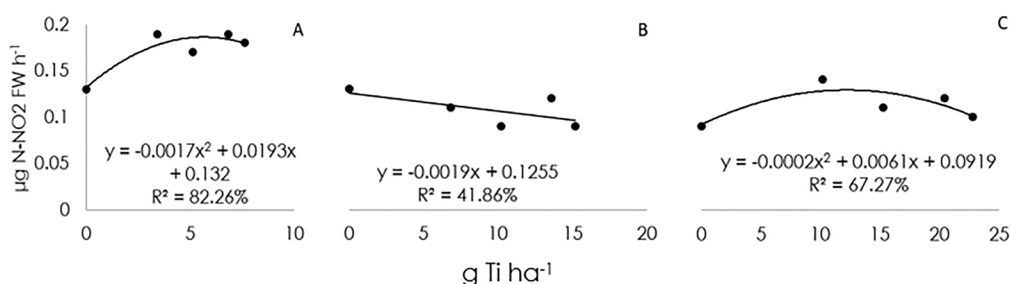
In the tuberization stage there was no effect of Ti levels on Mn content, ranging from 33.45 to 59.52 mg kg^{-1} DM. (Figure 3). In the tuber filling stage from 7.30 g of Ti ha^{-1} there was a positive influence on Mn contents in the leaves. This increase of Mn content in tuber filling stages possibly occurred as a function of the 'hormesis' effect of Ti.

Ti levels did not influence the Zn contents in the three stages, which ranged from 17.94 to 21.83; 15.59 to 22.71 and 17.94 to 20.73 mg kg^{-1} DM in plant growth, tuberization and tuber filling stages, respectively.
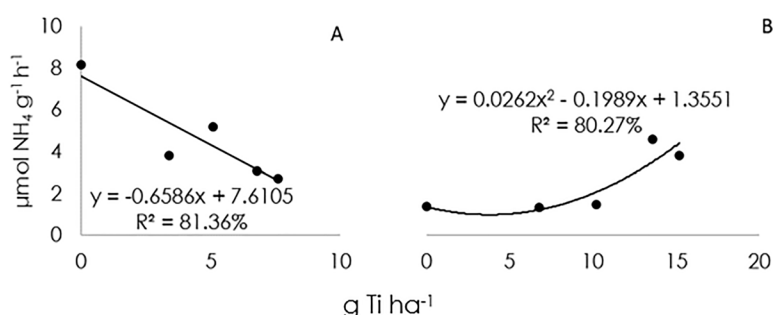
Enzyme activity

The activity of the enzyme nitrate reductase (ANR) increased as a function of Ti level up to 5.68 and 15.25 g of Ti ha^{-1}, respectively, in plant growth and tuber filling stages. In the tuberization stage, the activity of ANR decreased linearly with Ti increase (Figure 4), which can be explained by a substrate reduction for enzyme action, since in this stage it is normal to have a lower accumulation of N, and Fernandes (2011) verified that the lowest N accumulation in Agate cultivar occurs in the tuberization stage.

$$y = -0.0017x^2 + 0.0193x + 0.132 \quad R^2 = 82.26\%$$
$$y = -0.0019x + 0.1255 \quad R^2 = 41.86\%$$
$$y = -0.0002x^2 + 0.0061x + 0.0919 \quad R^2 = 67.27\%$$

Figure 4. Activity of enzyme nitrate reductase (ANR) in potato leaves as a function of Ti levels, in stages of plant growth (A), tuberization (B) and tuber filling (C).

In the growth stage, the activity of urease is inversely proportional to increasing levels of Ti, with a reduction of 65.8% in urease activity in the highest Ti level (7.6 g Ti ha^{-1}) (Figure 5). In the tuberization stage, the urease activity was initially reduced until the application of 3.8 g of Ti ha^{-1}, after which the activity of this enzyme was increased.
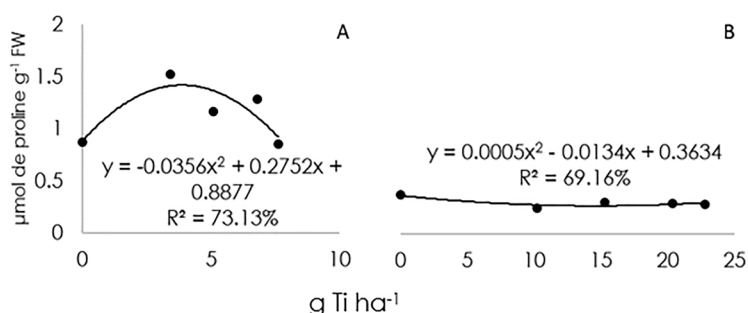

Figure 5. Activity of the enzyme urease in potato leaves as a function of Ti levels, in stages of plant growth (A) and tuberization (B).

In the tuber filling stage, urease values were found between 2.04 and 5.88 μmol NH$_4$ g^{-1} h^{-1} (Figure 5). Taking the hypothesis that Ti can inhibit Ni absorption, there is no specific information available in the literature regarding the absorption of Ni or its relation to other elements such as Ti.

The proline content in plant growth stage increased until 3.87 g ha^{-1}, there was no difference in tuberization stage, ranging from 0.65 to 1.38 μmol proline g^{-1} FW, and in the tuber filling stage there was a decrease up to 13.4 g ha^{-1} (Figure 6). This can be advantageous since too much synthesis of this amino acid can be detrimental to the plants, since it consumes between 0.4 and 0.6% of total N of the leaves (Ernst et al., 2000) and therefore the high proline synthesis can consume part of the N which could be used for filling tubers.

Figure 6. Proline content in potato leaves as a function of Ti levels, in stages of plant growth (A) and tuber filling (B).

Increasing levels of Ti up to 9.56 μmol de proline g^{-1} FW increased the action of superoxide dismutase enzyme (SOD) whereas in the tuber filling the increase was linear, in which the highest level promoted activity 3.5 times higher than when the Ti was not applied. In the growth stage the activity of the enzyme varied between 1.02 to 3.44 μg protein $^{-1}$ (Figure 7).
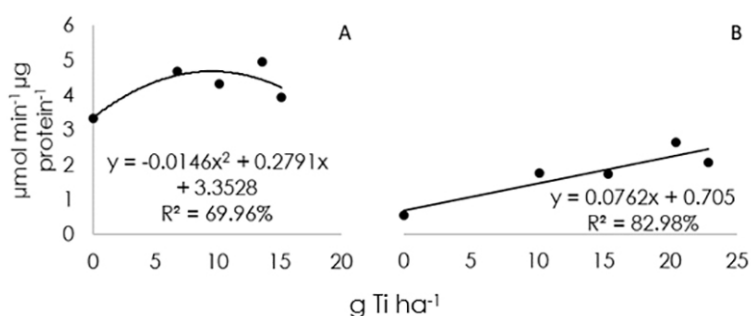
Figure 7. Activity of the enzyme superoxide dismutase (SOD) in potato leaves as a function of Ti levels, in stages of tuberization (A) and tuber filling (B).

SOD plays a key role in plant tolerance to oxidative stress, since it acts by transforming O^{2-} radicals into H_2O_2. Plants with high SOD activity have greater tolerance to oxidative stress. The activity of SOD depends on cofactors such as Cu, Zn, Fe and Mn. The reduction in Mn contents as a function of Ti application in the growth stage may have influenced the reduction of SOD activity.

The results corroborate with Song et al. (2013) who demonstrated a significant increase in SOD activity in tomato exposed to 200 mL of 5000 mg^{-1} of Ti under greenhouse conditions.

The activity of the enzyme peroxidase (POD) showed an increase with Ti levels with an increase in plant growth stage. In the other stages, the activity varied between 0.17 to 0.22 µmol min^{-1} µg protein $^{-1}$ and 0.34 to 0.43 µmol min^{-1} µg protein $^{-1}$ in tuberization and tuber filling, respectively (Figure 8). Carvajal et al. (1994) also verified higher activity of POD in pepper plants (*Capsicum annuum*).

The activity of the enzyme catalase (CAT) increased until 5.64 g Ti ha^{-1}, in plant growth stage. In tuberization stage there was an opposite behavior, with the activity reduced until 19.31 g Ti ha^{-1} followed by an increase with Ti application (Figure 9). Carvajal et al. (1994) also observed a linear increase in the activity of CAT enzyme as a function of Ti application in peppers (*Capsicum annuum*).
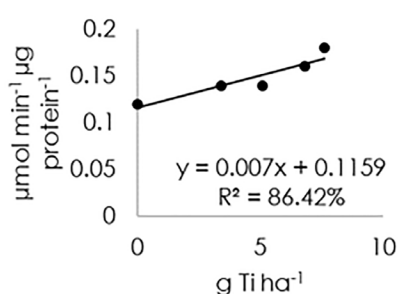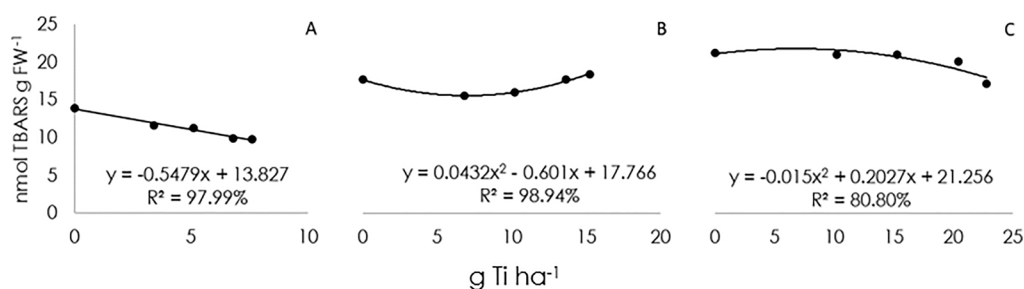

Figure 8. Activity of peroxidase (POD) in potato leaves as a function of Ti levels, in stages of plant growth.
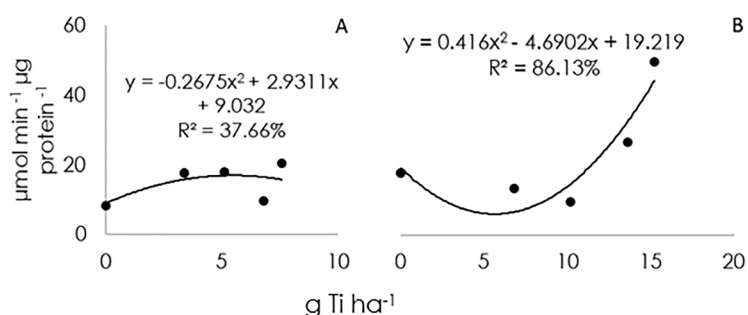
Figure 9. Activity of the enzyme catalase (CAT) in potato leaves as a function of Ti levels, in stages of plant growth (A) and tuberization (B).

The lipid peroxidation (PL) in plant growth stage was decreasing with Ti increase, in tuberization stage the PL decreased to the level of 6.96 g Ti ha^{-1}, but there was an increase from this level. At the tuber filling stage, there was a reduction from the level of 6.76 g Ti ha^{-1} (Figure 10).


Figure 10. Activity of lipid peroxidation (PL) in potato leaves as a function of Ti levels, in stages of plant growth (A), tuberization (B) and tuber filling (C).

The increase of free radical production is indicated as a toxicity mechanism of several metals. The PL in treatment with Ti in plant growth and tuberization stages were kept below the values observed in the treatments without application of Ti and thus it can be inferred that there was no toxicity caused by Ti levels.

Productivity

The mean matter (MMT) and mean diameter of tubers (DMT) were not affected by Ti levels, varying between 73.14 to 80 g and 41 and 43.5 mm, respectively. The productivity was affected by foliar application of Ti (Figure 11). The level for maximum tuber production was 11.3 g of Ti ha^{-1} at a yield of 32.7 t ha^{-1}.

Inside figure:
$$Y = -0.04x^2 + 0.98x + 27.22$$
$$R^2 = 54.95\%$$
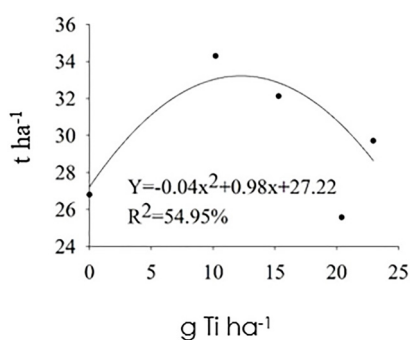
Figure 11. Productivity of potatoes (t ha^{-1}) as a function of Ti levels.

In comparison to absence of Ti application, this productivity represented an increase of 20.4%. Carvajal et al. (1994) obtained pepper yield increases of 32% in relation to plants that were not treated with Ti. These authors emphasized that the effect of Ti on plants depends essentially on Ti concentrations.

In addition, Ti nanoparticles also showed satisfactory results in the yield of tomato (Raliya et al., 2015a, b), demonstrating Ti could be a beneficial alternative to improve managements and production.

Conclusions

Ti positively affects potato development, as observed in the present study, through its action on enzyme activity and increase in productivity. Therefore, the nutrient has the potential to be used to improve production, being another alternative to increase the sustainability of crops.

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