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Copper oxychloride fungicide and its effect on growth and oxidative stress of potato plants

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ABSTRACT

Excess copper in plants causes physiological alterations that lead to crop productivity losses. However, cupric fungicides have been utilized in the control of *Alternaria solani* and *Phytophtora infestans* fungi, which cause early blight and late blight in potato, respectively. Thus, this study aimed to investigate the effect of different copper oxychloride levels on potato plants through some biochemical and physiological parameters. The fungicide was applied at the recommended level (2.50 g L^{-1}), at a reduced level (1.25 g L^{-1}), and at 5.00 g L^{-1}, to simulate spraying in the field twice during the same period with the recommended level. The results revealed that superoxide dismutase (SOD, EC 1.15.1.1) protected plants against oxidative stress at the beginning of the cycle since lipoperoxide levels were low in that period. In addition, increased SOD activity positively correlated with increased usable leaf area for photosynthesis (leaf area ratio, LAR), photosynthetic effectiveness (net assimilation rate, NAR), and growth relative to pre-existing dry matter (relative growth rate, RGR). Concomitantly, there was a negative correlation between lipoperoxide levels and LAR and RGR. Plants randomly sprayed twice in the same period with the level recommended for potato crop protection in the field do not present damage regarding their development. However, additional studies are needed in order to reduce the use of copper fungicides in the control of early and late blight in potato crop production, then decreasing the release of copper in the environment.

1. Introduction

Copper oxychloride (3Cu $(OH)_2$.$CuCl_2$) is a fungicide used at 2.50 g L^{-1} against early and late blight in potato crops [1]. Although copper is essential for metabolic processes in all organisms when in trace amounts, the use of copper-based fungicides has been ecologically harmful [2]. Hence, a reduction or replacement of copper compounds in disease control is desirable [3]. In organic farming, a shift towards the use of blight resistant potato varieties should be strongly encouraged, although this is unlikely to eliminate the need to use copper fungicides [4].

Excess copper can produce reactive oxygen species (ROS) such as superoxide anion radical (O_2^-), hydroxyl radicals (OH*) and hydrogen peroxide (H_2O_2) [5] and can trigger an important reorganization of the root system architecture [6]. In plants, ROS are highly reactive and toxic and cause damage to proteins, lipids, carbohydrates and DNA which ultimately results in oxidative stress [7]. Lipoperoxide formation, a consequence of ROS production, is an indicator of the oxidative stress level in plants [8]. Rises in lipoperoxide levels have been reported in *Lessonia nigrescens* subjected to copper [9].

Naturally, plants have a ROS-scavenge antioxidant defense system that provides protection against oxidative stress, including the enzymes glutathione S-transferase (GST, EC 2.5.1.18) and

Abbreviations: BSA, bovine serum albumin; CDNB, 1-chloro-2,4-dinitrobenzene; CEC, cation exchange capacity; DAP, days after planting; EDTA, ethylenediaminetetraacetic acid; GSH, glutathione; GST, glutathione S-transferase; LAR, leaf area ratio; NAR, net assimilation rate; NBT, nitro blue tetrazolium; OM, organic matter; PVPP, polyvinylpolypyrrolidone; RGR, relative growth rate; ROS, reactive oxygen species; SB, sum of bases; SLA, specific leaf area; SOD, superoxide dismutase; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; TCA, trichloracetic acid; TRIS, tris (hydroxymethyl) aminomethane.

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superoxide dismutase (SOD, EC 1.15.1.1), from which the activity can indicate such stress. SOD is widely distributed among O_2-consuming organisms and is responsible for the dismutation of O_2^- into H_2O_2, therefore influencing the concentration of O_2^- and H_2O_2 [10]. Glutathione S-transferases (GSTs) are an isozyme family, which catalyze the conjugation of the reduced tripeptide glutathione (GSH) to a variety of hydrophobic and electrophilic substrates [11].

Copper oxychloride can affect plant productivity and yield. Plant productivity evaluation is important in such situations of unbalanced copper levels and can be done through monitoring productivity dynamics [12] by using leaf area ratio (LAR), which expresses usable leaf area for photosynthesis; specific leaf area (SLA), which indicates the inverse of leaf thickness; net assimilation rate (NAR), which reflects net photosynthesis; and relative growth rate (RGR), which represents the increase relative to dry matter at the moment the observation period begins.

Soils may contain elevated levels of copper because of its widespread use as a pesticide, land application of sewage sludges as well as mining and smelting activities [13]. Since copper has a low mobility in soil, it may tend to accumulate in the upper soil layers [14]. In the field, plants can be randomly sprayed twice in the same period with the recommended level of pesticides. So, this study aimed to investigate the effect of copper oxychloride sprayed on potato plants at the recommended level (2.50 g L^{-1}), at a reduced level (1.25 g L^{-1}) and twice the recommended level (5.00 g L^{-1}). Plants were evaluated as to leaf area, leaf blade dry matter, tuber dry matter yield, total dry matter, physiological indexes from the growth analysis, lipoperoxide levels, SOD and GST activity, and copper accumulation in dry matter.

2. Materials and methods

2.1. Plant material and experimental design

Seed tubers of potato (*Solanum tuberosum* ssp *tuberosum* L.) cultivar Mondial showing emerging sprouts were used. The experiments were carried out in a greenhouse at Botucatu (22°53′09″ S; 48°26′42″ W), State of São Paulo, Brazil. Plants were grown in 17L pots (34 cm external diameter and 31 cm height). Each pot was filled with dirt from a soil classified as Structured Red Nitosol, which presented the following characteristics: sand: 25%, silt: 18%, clay: 57%, OM: 2.7%, CEC: 89 eq mg 100 cm^{-3}, and SB: 57%. After liming, the dirt had the following characteristics and composition: pH ($CaCl_2$) 5.6, P: 114, B: 0.61, Cu: 1.2, Fe: 47, Mn: 2.6, Zn: 3.4 (mg dm^{-3}), H + Al: 20, K: 2.3, Ca: 57, Mg: 22, Al^{3+}: 0, SB: 81, CEC: 102, V%: 80 ($mmol_c$ L^{-1} dm^{-3}), and OM: 25 g dm^{-3}. Two seed tubers were planted in each pot, which were then considered a sampling unit. Macroscopic evaluations and irrigations were performed daily. The experimental design consisted of randomized blocks, with four replicates, in a 4 × 5 factorial arrangement, i.e., four copper oxychloride levels (0.00, 1.25, 2.50, and 5.00 g L^{-1}), and five harvests at seven-day intervals: 27, 34, 41, 48, and 55 days after planting (DAP).

2.2. Copper oxychloride application

Copper oxychloride was applied by using a compressed-air spraying system equipped with nozzles on a movable bar, at controlled speed and pressure (2.50 kgf cm^{-2}). In the first and second applications, the volume was equivalent to 200 L ha^{-1}, using XR 110.02 nozzles. In the remaining applications, due to higher plant size, the applied volume was equivalent to 400 L ha^{-1}, using XR 110.04 nozzles. Treatments were begun when plants reached a mean height of 15 cm and had fully-expanded leaves, after the seed tubers had been planted in the pots.

2.3. Leaf blade sampling for biochemical assays

At all harvests, five leaf blades from each sampling unit were collected, weighed, packed, frozen in liquid nitrogen and stored at −80 °C for later biochemical evaluations, including determination of lipoperoxide levels, as well as SOD and GST activities.

2.4. Lipoperoxide content

To measure lipoperoxide content, leaf blades were ground with 5 mL of a solution containing 0.25% thiobarbituric acid (TBA) and 10% trichloracetic acid (TCA). The mixture was heated at 90 °C for 1 h, quickly cooled, and then centrifuged at 10,000*g* for 15 min. The absorbance of the supernatant was read at 560 and 600 nm. The malondialdehyde absorbance coefficient (155 mmol L^{-1} cm^1) was used for the calculations. Results were expressed as nmol thiobarbituric acid reactive substances (TBARS) g^{-1} fresh matter [15].

2.5. Enzymatic extraction

To obtain enzymatic extracts, the method described by Ekler et al. [16] was used. Leaf blade samples were ground in a cold mortar with 5 mL of 0.2 mol L^{-1} tris (hydroxymethyl) aminomethane-hydrochloric acid (TRIS–HCl) cold buffer pH 7.8 containing 1 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA), 7.5% (w v^{-1}) polyvinylpolypyrrolidone (PVPP) and a small amount of washed sterile sand. After centrifugation at 4 °C (20 min at 14,000*g*), the supernatant was collected and stored at −20 °C until used in the determination of SOD and GST activities and soluble protein content, as well as in the quantification of the specific activity of these enzymes.

2.6. SOD assay

SOD activity was determined according to the method of Beauchamp and Fridovich [17]. In brief, the adopted reaction system was 50 mmol L^{-1} sodium phosphate buffer pH 7.8, 33 μmol L^{-1} nitro blue tetrazolium (NBT) + 0.66 mmol L^{-1} EDTA (5:4) mixture, 10 mmol L^{-1} L-methionine + 0.0033 mmol L^{-1} riboflavin (1:1) mixture and enzymatic extract, in a volume of 3.0 mL. It was kept at 25 °C under light for 10 min. One enzymatic unit (U) of SOD, expressed as U mg^{-1} protein, was defined as the enzyme quantity needed to cause 50% inhibition in the NBT reduction rate, measured at 560 nm.

2.7. GST assay

GST activity was assessed according to the conditions cited by Wu et al. [18]. The reaction system contained the enzymatic extract, 100 mmol L^{-1} potassium phosphate buffer pH 6.9, 3.3 mmol L^{-1} glutathione (GSH), and 30 mmol L^{-1} 1-chloro-2, 4-dinitrobenzene (CDNB), in a total volume of 3.0 mL. It was kept at 25 °C for 30 min. Absorbance change due to the formation of GSH-CDNB conjugate was measured in a spectrophotometer at 340 nm. A molar extinction coefficient equal to 10 mmol L^{-1} cm^{-1} [19] was used to calculate the enzyme specific activity, expressed as mmol L^{-1} min^{-1} mg^{-1} protein.

2.8. Soluble protein content

Soluble protein content of the enzymatic extracts, which is employed in the assessment of SOD and GST specific activities,

was estimated through the method of Lowry et al. [20] using bovine serum albumin (BSA) as standard.

2.9. Leaf area and dry matter measurement

In addition to the biochemical evaluations, at each harvest the evaluated plants were separated into leaf blades, stems plus petioles, roots, and tubers when present. Then, leaf area (dm^2) was measured in a leaf area meter model Li-COR 300 and defined as the sum of the areas of all leaf blades from the plant. The various organs were separately packed in labeled paper bags and dried in a forced aeration oven, between 60 and 70 °C, until obtaining a constant matter value (g), which was then measured in an analytical scale of 0.1 mg readability. Total dry matter corresponded to the sum of matter values from all existing organs in each harvest and each treatment.

2.10. Copper content

For copper level quantification, 0.5 g dry matter of tubers, roots, stems plus petioles, and leaf blades harvested at 55 DAP were subjected to a wet digestion pretreatment with nitric and perchloric acids (3:2, pH < 2.0) at 130–150 °C for approximately 30 h in order to discard all organic matter [21]. Copper levels were then assessed by using an atomic absorption spectrophotometer [22].

2.11. Physiological indexes evaluation

The physiological indexes leaf area ratio (LAR), specific leaf area (SLA), net assimilation rate (NAR) and relative growth rate (RGR) were evaluated based on the adjustment of the variables leaf area, leaf blade dry matter and total dry matter of plants in relation to time, i.e. age of plants. This was done through a cubic exponential equation (Table 1).

2.12. Statistical analysis

The variables leaf area, leaf blade dry matter, tuber dry matter yield, total dry matter, copper levels in tubers, roots, stem plus petioles and leaf blades, lipoperoxide levels, and SOD and GST activities were subjected to an *F*-test through analysis of variance at 0.05 significance by using the SAS software [23]. The homogeneity of variances was verified through Levene's test [24] also by using the SAS software [23]. The original data from the evaluated variables presented homogeneous variances, except for lipoperoxide levels and SOD activity. Thus, the results regarding both variables were transformed to square root, which homogenized their variances. Then, polynomial regression models were developed when the interaction copper oxychloride levels × days after planting (DAP) was significant. For the harvest periods in which this interaction was not significant, only points related to each fungicide level were represented in the graphs. When this interaction was not significant in any harvest, as observed for total dry matter, the model expresses the mean values of all harvest periods at each fungicide level. Polynomial regression models were developed based on the considerations mentioned by Gomes [25]. According to the author, when there is no independence of several treatments evaluated, the analysis of variance must reflect the dependence among treatments otherwise it will not be valid. This procedure is suitable in cases of quantitative treatments (e.g. increasing levels of fertilizers or insecticides), thus justifying the existence of a functional correspondence (named regression equation) that connects the values of treatments (X) to those analyzed (Y). Also in the current study, the obtained physiological indexes from the growth analysis and oxidative stress results were subjected to Spearman's non-parametric correlation test at 5% significance.

3. Results and discussion

3.1. Lipoperoxide content and activity of SOD and GST

There was interaction between copper oxychloride level and DAP for lipoperoxide levels, SOD activity and GST activity (Fig. 1). At 27 DAP, the lowest lipid peroxidation and the highest SOD activity were recorded, which suggests SOD protected potato plants against oxidative stress at the beginning of the cycle. Chi Yu et al. [5] and Drazkiewicz et al. [26] also observed an increase in SOD activity in rice leaves and *Arabidopsis thaliana*, respectively, both treated with copper sulphate, In the present study, although

Table 1
Cubic exponential equations and R^2 values for the physiological indexes LAR, SLA, RGR and NAR in *Solanum tuberosum* ssp *tuberosum* L. cv. Mondial plants treated with different copper oxychloride levels.

g L^{-1}[a]	LAR[b] ($dm^2\ g^{-1}$)	SLA[c] ($dm^2\ g^{-1}$)	RGR[d] ($g\ g^{-1}\ day^{-1}$)
0.00	$3.51E+03e^{(-0.53t+1.11E-02t2-8.08E-05t3)}$	$2.77E+04e^{(-0.72t+1.79E-02t2-1.45E-04t3)}$	$1.27-5.66E-02t+6.19E-04t^2$
1.25	$6.47e^{(-0.03t-1.08E-03t2+1.61E-05t3)}$	$1.10e^{(0.07t-1.49E-03t2+7.08E-06t3)}$	$0.74-3.25E-02t+3.48E-04t^2$
2.50	$13.5e^{(-0.12t+2.08E-03t2-1.85E-05t3)}$	$53.4e^{(-0.15t+2.80E-03t2-2.09E-05t3)}$	$0.43-1.73E-02t+1.80E-04t^2$
5.00	$1.82E+10e^{(-1.73t+4.15E-02t2-3.27E-04t3)}$	$1.04E+03e^{(-0.43t+1.04E-02t2-8.51E-05t3)}$	$0.78-3.31E-02t+3.49E-04t^2$
g L^{-1}[a]	NAR[e] ($g\ dm^{-2}\ day^{-1}$)		
0.00	$(1.27-5.66E-02t+6.19E-04t^2)\ 5.35E-08e^{(1.27t-2.83E-02t2+2.06E-04t3)}/1.88E-04e^{(0.74t-1.72E-02t2+1.26E-04t3)}$		
1.25	$(0.74-3.25E-02t+3.48E-04t^2)\ 9.67E-05e^{(0.74t-1.62E-02t2+1.16E-04t3)}/6.26E-04e^{(0.71t-1.73E-02t2+1.32E-04t3)}$		
2.50	$(0.43-1.73E-02t+1.80E-04t^2)\ 4.15E-03e^{(0.43t-8.67E-03t2+6.00E-05t3)}/5.61E-02e^{(0.31t-6.59E-03t2+4.14E-05t3)}$		
5.00	$(0.79-3.31E-02t+3.49E-04t^2)\ 2.56E-05e^{(0.79t-1.65E-02t2+1.16E-04t3)}/4.66E+05e^{(-0.95t+2.49E-02t2-2.10E-04t3)}$		
g L^{-1}[a]	R^2 (TDM)[f]	R^2 (LAI)[g]	R^2 (LBDM)[h]
0.00	0.948	0.963	0.994
1.25	0.914	0.990	0.901
2.50	0.999	0.972	0.997
5.00	1.000	1.007	0.962

TDM and LAI: adjusted for LAR. NAR and RGR; LBDM and LAI: adjusted for SLA.
[a] g L^{-1}: copper oxychloride levels;
[b] LAR: leaf area ratio;
[c] SLA: specific leaf area;
[d] RGR: relative growth rate;
[e] NAR: net assimilation rate;
[f] TDM: total dry matter;
[g] LAI: leaf area index;
[h] LBDM: leaf blade dry matter.

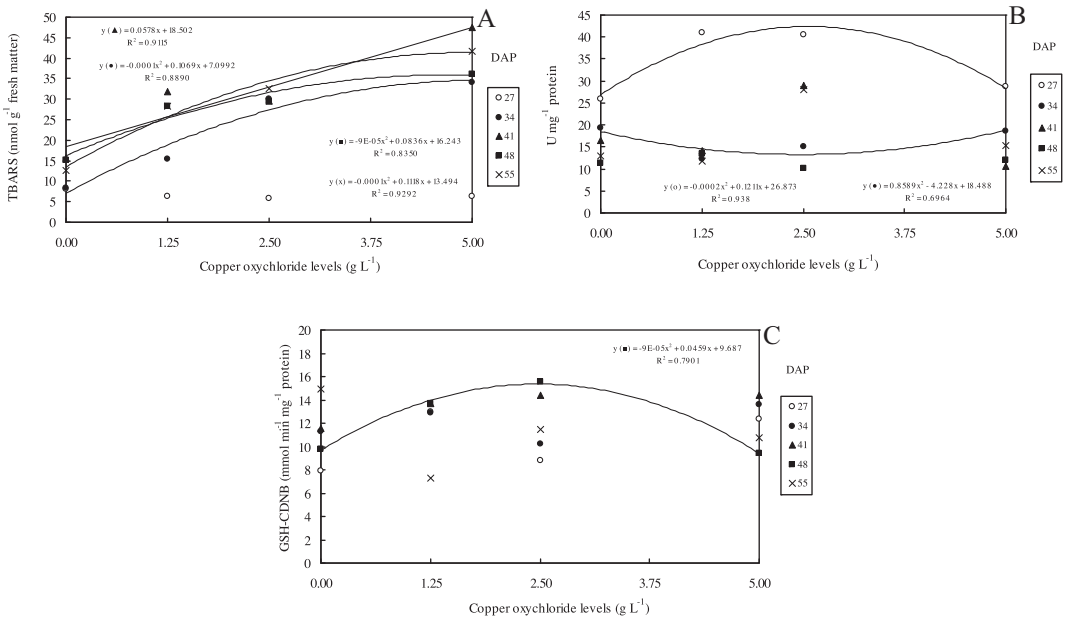

Fig. 1. (A) Lipoperoxide levels (nmol TBARS g^{-1} fresh matter); (B) Superoxide dismutase (SOD, U mg^{-1} protein) activity; (C) Glutathione S-transferase (GST) activity of *Solanum tuberosum* ssp *tuberosum* L. cv Mondial plants treated with different copper oxychloride levels, at 27, 34, 41, 48, and 55 days after planting (DAP). Means of four replicates. TBARS: thiobarbituric acid reactive substances. SOD activity is expressed as U mg^{-1} protein. One SOD enzymatic activity unit (U) represents the amount of enzyme needed to cause 50% inhibition in NBT reduction ratio. GST activity is expressed as mmol min^{-1} mg^{-1} protein of produced GSH-CDNB complex.

there was no copper oxychloride effect at 55 DAP, GST activity tended to decrease in fungicide-treated plants (Fig. 1C), which can be due to their high lipoperoxide levels (Fig. 1A). Conversely, Smith et al. [27] observed the induction of four GSTs (*At*GSTF2, *At*GSTF6, *At*GSTF7, and *At*GSTU19) in copper-treated *A. thaliana* seedlings. Also, Khatun et al. [28] reported that copper treatment gradually activated GST with increasing copper concentrations (up to 200 μM) in Indian ginseng (*Withania somnifera* L. Dunal) plants. Based on control results, herein, the level of 5.00 g L^{-1} probably overloaded part of the antioxidant system represented by SOD and GST. Since higher lipoperoxide levels were observed, without interfering with the plant development, other antioxidants may have acted.

3.2. Leaf area, leaf blade dry matter, tuber dry matter, total dry matter and copper content

The interaction between copper oxychloride level and DAP concerning leaf area was detected at 48 and 55 DAP (Fig. 2A). There was interaction between copper oxychloride level and DAP in the analysis of leaf blade dry matter (Fig. 2B) and tuber dry matter yield (Fig. 2C). Such interaction was not detected in total dry matter (Fig. 2D).

At 48 DAP, leaf area increased with increasing copper oxychloride levels (Fig. 2A). At 55 DAP, plants treated with 5.00 g L^{-1} tended to present higher leaf areas. However, Vinit-Dunand et al. [29] found decreased leaf areas in young leaves of cucumber (*Cucumis sativus* L.) kept in nutrient solution with additional supplementation of 10 μg g^{-1} Cu. In the present study, plants subjected to 5.00 g L^{-1} presented increased copper levels in leaf blades (Fig. 3D), which may have been responsible for the increase of leaf area and leaf blade dry matter (Fig. 2A and B). Nevertheless, Alaoui-Sossé et al. [13] studied twenty-day-old cucumber plants submitted to copper stress during 5 days and observed that leaf expansion rather than dry matter accumulation was the first target of copper inhibition.

The lowest tuber dry matter production was recorded in plants exposed to 2.50 g L^{-1} at 41 and 48 DAP (Fig. 2C). Among fungicide-treated plants, those subjected to 5.00 g L^{-1} tended to present increasing values in the above-mentioned periods, similarly to controls. As to copper levels in tissues, in general the successive fungicide applications led to higher copper accumulation in leaf blades, followed by stems plus petioles, roots, and tubers, in a directly proportional manner to the sprayed level (Fig. 3). Such fact demonstrated that copper translocation is low in potato plants. This observation was corroborated by Loneragan [30], who stated that copper is generally considered phloem immobile unless it is linked to senescence whereupon it becomes mobile. In leaves, heavy metals are absorbed by the epidermis, transported across the plasma membrane of cells and immobilized within the cells [31]. However, in the current study fungicide-treated plants had higher copper levels in tubers relative to untreated ones (Fig. 3A). It must be emphasized that such levels are above the maximum limit (10 mg kg^{-1}) allowed by the Brazilian National Health Surveillance Agency [32]. Srek et al. [33] investigated the tuber yield, copper concentration in tubers and its uptake in potato plants subjected to the treatment with pig slurry mixed with straw, which contained 406 g ha^{-1} Cu. The authors observed that Cu uptake was clearly determined by tuber yield and was relatively independent of the amount of Cu supplied, which did not corroborate the results of the present study.

3.3. Physiological indexes

LAR reflects the usable leaf area for photosynthesis. This index constantly decreased throughout the cycle up to the level of 2.50 g L^{-1} (Fig. 4A). Similar results were observed by Bertani [34], who studied potato plants under different phosphoric irrigation depths. Since leaf area is responsible for luminous energy interception and CO_2, and total dry matter results from photosynthesis, Benincasa [12] stated that LAR may decrease at a certain stage as a consequence of an increased interference of higher leaves over lower ones, which can lead to self-shading. On the other hand, in the present study plants treated with 5.00 g L^{-1} had an initial decrease in LAR, followed by an increase up to 48 DAP then a new decrease at 55 DAP. Those plants presented higher leaf area

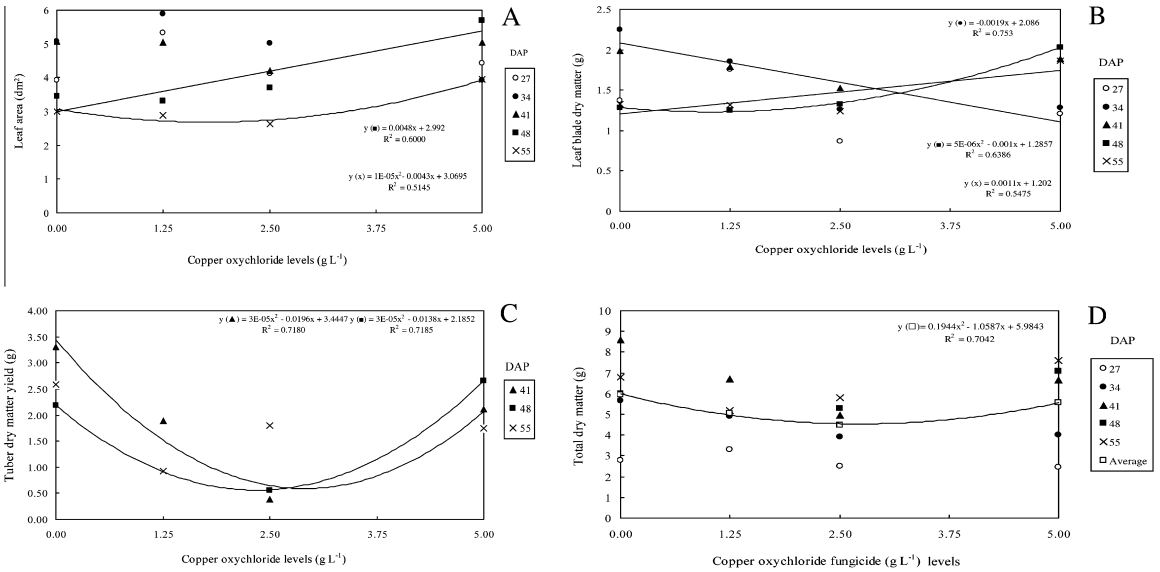

Fig. 2. (A) Leaf area (dm^2); (B) Leaf blade dry matter (g); (C) Tuber dry matter yield (g); (D) Total dry matter (g) of *Solanum tuberosum* ssp *tuberosum* L. cv Mondial plants treated with different copper oxychloride levels. A, B and D, harvests at 27, 34, 41, 48, and 55 days after planting (DAP). C, harvests at 41, 48 and 55 DAP. Means of four replicates.

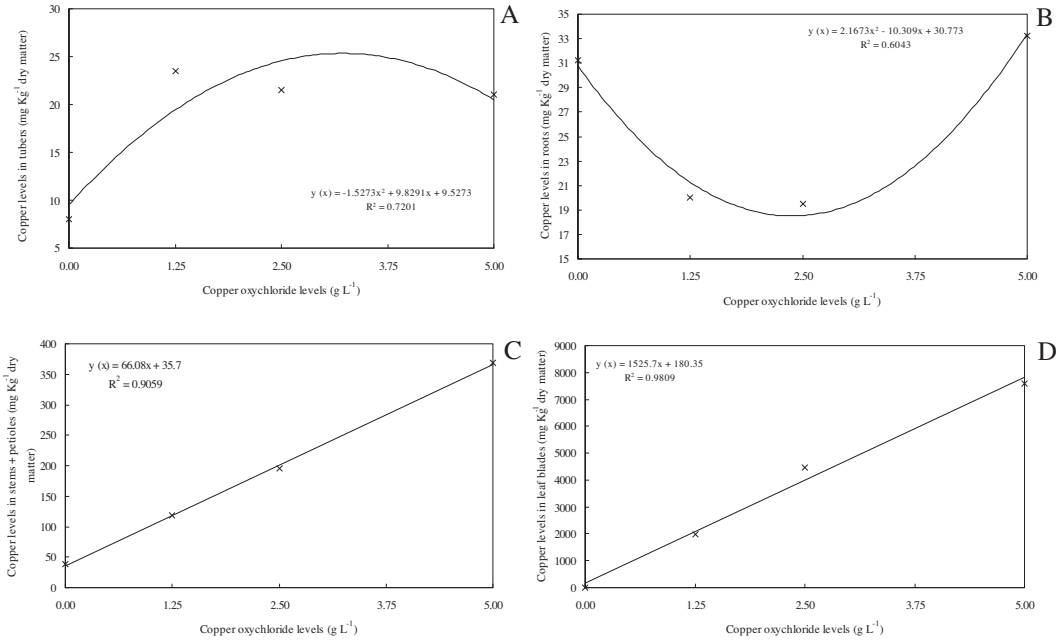

Fig. 3. Copper levels (mg kg^{-1}) in tubers (A), roots (B), stems + petioles (C) and leaf blades (D) of *Solanum tuberosum* ssp *tuberosum* L. cv. Mondial plants treated with different copper oxychloride levels, at 55 days after planting. Means of four replicates.

and leaf blade dry matter at 48 and 55 DAP, relative to plants in the other treatments. This fact suggests that the highest fungicide level stimulated plant resprouting, which may have led to an increase in usable leaf area for photosynthesis, justifying the LAR increase verified in those plants between 34 and 48 DAP.

At the beginning of the cycle, plants treated with 2.50 g L^{-1} had higher SLA. In these plants and in those treated with 1.25 and 5.00 g L^{-1}, which had lower SLA variation, this index showed constant decrease throughout the cycle (Fig. 4B). SLA values can be higher at the beginning of development, which reveals plants with few thick leaves, little dry matter, and small leaf area [12]. Bertani [34] reported that at the beginning of vegetative development, SLA values are higher; then, they decrease and remain constant. In the present study, during tuber production, SLA values, which reflect the inverse of leaf thickness, steadily decreased in treated plants, although not very markedly. Consequently, those plants had an increase in leaf thickness during the tuber production period. This result may be due to a greater efficiency in material accumulation, precisely when translocation of photoassimilates to the tubers occurs [34].

NAR represents the photosynthetic efficiency, whereas RGR reflects growth based on pre-existing material. Both indexes

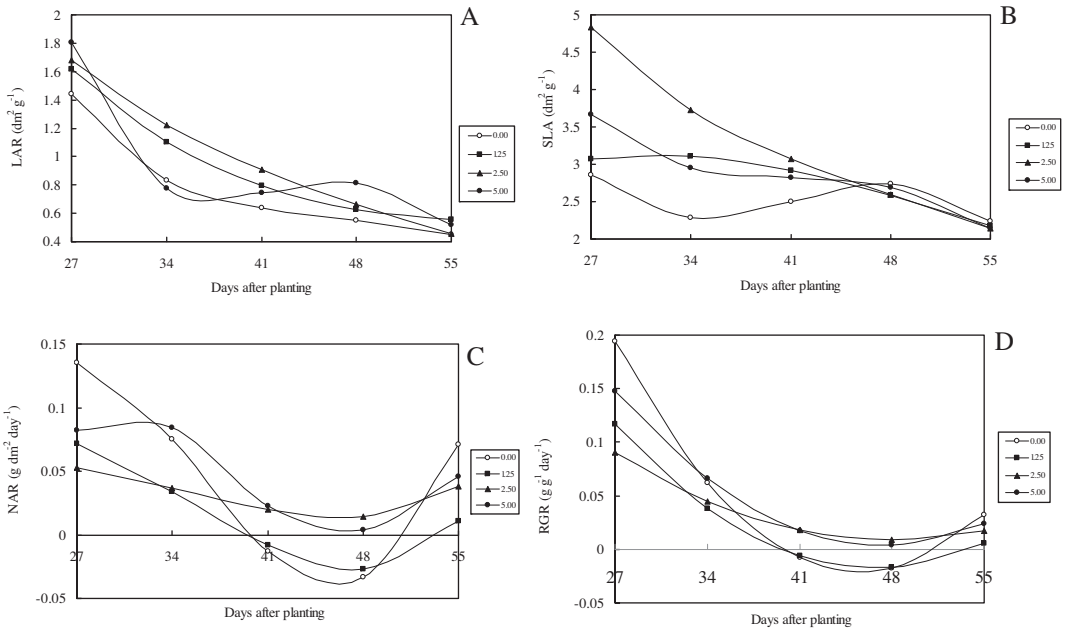

Fig. 4. (A) Leaf area ratio (LAR), $dm^2\ g^{-1}$; (B) Specific leaf area (SLA), $dm^2\ g^{-1}$; (C) Net assimilation rate (NAR), $g\ dm^{-2}\ day^{-1}$; (D) Relative growth rate (RGR), $g\ g^{-1}\ day^{-1}$ of *Solanum tuberosum* ssp *tuberosum* L. cv Mondial plants treated with different copper oxychloride levels (0.00, 1.25, 2.50, and 5.00 g L^{-1}), in several harvests. Means of four replicates. Values were fit through a cubic exponential equation (Table 1).

decreased at the beginning of the cycle (Fig. 4C and D). NAR increase was more evident at the end, i.e. the production period, probably due to resprouting. Bertani [34] stated that during translocation of photoassimilates to tubers, the potato plant increases material accumulation efficiency. This result could be confirmed in the present study in plants treated with 2.50 g L^{-1}, which had low and constant NAR and RGR values throughout the cycle, as well as the lowest tuber dry matter yield at 41 and 48 DAP. Low NAR and RGR values during the plant's life cycle indicate slower growth [34]. Thus, the high SLA presented by those plants at the beginning of the cycle resulted in low NAR throughout the cycle, which indicates that the leaves did not become very thick, coinciding with low tuber dry matter yield. Bertani [34] observed higher NAR at the initial developmental stage, which later decreased and finally increased at the end of the study period in all treatments – the same NAR result verified in the present work, although the methodologies were different.

3.4. Correlation test between physiological indexes and oxidative stress results

SOD protected potato plants against oxidative stress at the beginning of the cycle since lipoperoxide levels were low in that period. The increase of SOD activity, thus indicating its protective action, positively correlated with the increase of the usable leaf area for photosynthesis (LAR), photosynthetic effectiveness (NAR), and growth relative to pre-existing dry matter (RGR). Concurrently, there was a negative correlation between lipoperoxide levels and the indexes LAR and RGR (Table 2). Within a cell, SODs constitute the first line of defense against ROS [35,36]. ROS are capable of chemically altering the main biomolecule classes, leading to structural and functional changes in lipids, proteins, chlorophylls, and nucleic acids [37,38]. Among such alterations are chlorophyll breakdown, DNA fragmentation, ion leakage, lipid peroxidation, and cell death [39]. Based on these considerations, in the present study we can infer that the positive correlation between increased SOD activity and growth parameters can be due to the high maintenance of cell homeostasis due to O_2^- scavenging by SOD.

Table 2
Spearman correlation coefficients between physiological indexes (LAR, SLA, NAR, RGR) and oxidative stress results (GST, SOD, lipoperoxides) in *Solanum tuberosum* ssp *tuberosum* L. cv. Mondial plants treated with different copper oxychloride levels.

	GST[e]	SOD[f]	Lipoperoxides
LAR[a]	−0.10526	0.52030*	−0.45865*
SLA[b]	0.09624	0.33233	−0.30827
NAR[c]	−0.08271	0.58797*	−0.32782
RGR[d]	−0.09252	0.63483*	−0.44829*

[a] LAR: leaf area ratio;
[b] SLA: specific leaf area;
[c] NAR: net assimilation rate;
[d] RGR: relative growth rate;
[e] GST: glutathione S-transferase;
[f] SOD: superoxide dismutase.
* Significant at 5%.

3.5. Conclusions

The highest fungicide level, 5.00 g L^{-1}, positively affected plant growth at the end of the cycle, leading to increases in leaf area and leaf blade dry matter, which probably allowed such plants to tend to present the best tuber dry matter yield relative to those treated with 1.25 and 2.50 g L^{-1}. At 55 DAP, copper oxychloride did not interfere with tuber dry matter yield, although higher copper levels were detected in tubers from fungicide-treated plants in that period. When plants were sprayed with the recommended level, 2.50 g L^{-1}, plant growth was slower over the cycle, which was confirmed by the lower decreases in NAR and RGR curves, together with the lowest tuber dry matter yield values at 41 and 48 DAP. Thus, although the recommended level of copper oxychloride for potato crop corresponds to 2.50 g L^{-1}, under the conditions used in the present work, plants treated with 5.00 g L^{-1} were less influenced by the presence of fungicide. This was probably due to their resprouting at the end of the cycle, since LAR, NAR, leaf areas, and leaf blade dry matter were higher in that period, i.e. the production period. Although such plants had higher lipoperoxide levels, their development was not affected, probably due to an antioxidant action of other enzymes or non-enzymatic compounds, since SOD

exhibited protective action at the beginning of the potato plant cycle. Hence, plants randomly sprayed twice during the same period with the level recommended for potato crop protection in the field do not present damage regarding their development. However, additional studies are needed in order to reduce the use of copper fungicides in the control of early and late blight in potato crop production, then decreasing the release of copper in the environment.

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