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Deciphering Ciprofloxacin's Impact on Growth Attributes and Antioxidant Compounds in Pasankalla Quinoa

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Abstract: The utilization of irrigation water contaminated with antibiotics can potentially affect the growth and production of metabolites in crops. Thus, the effect of Ciprofloxacin (CIP) in irrigation water on the morphological characteristics and polyphenol content in the Pasakalla variety of quinoa was evaluated. Quinoa seeds were sown and irrigated twice weekly with different CIP doses (1, 10, and 100 µg/L). The plant was divided into roots, leaves, panicles, and grain to measure their morphological characteristics and antioxidant properties (the content of total polyphenols, antioxidant capacity, and polyphenolic profile). Root length and biomass of the plant were not affected by the dosage of CIP during physiological maturity. On the contrary, plant growth was reduced (16%) when 10 ug/L of CIP was used. On the other hand, the presence of high CIP concentrations (100 ug/L) improved the biosynthesis of polyphenols and antioxidant capacity by 52 and 59% compared to the control. Profile polyphenols show that vanillic acid and procyanidins A2 and B2 were significantly increased. Thus, the use of CIP could promote a higher enzymatic activity to produce specific polyphenols in order to inhibit the presence of ROS. Finally, under CIP-controlled conditions, the production of polyphenols could be improved without altering the plant's expected growth.

Keywords: antibiotics; pasankalla quinoa; morphological characteristic; antioxidant compounds

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1. Introduction

Peru produces 98,000 tons of grains from quinoa (*Chenopodium quinoa* Willd) per year, where ~46% of this production is destined for the international market [1]. Thus, different varieties of quinoa have been developed in the high Andean regions (Puno, Cusco, and Junin) like Negra-Collana, Pasankalla, Salcedo-INIA, Quillahuaman, Amarilla, and Junín [2]. In particular, Pasankalla is a pseudocereal cultivated in Puno with important nutritional and bioactive properties [2,3]. For example, this grain presents 14% proteins, 14.3% dietary fibers, and 7% lipids; the total polyphenol content can vary between 60 and

65 mg GAE/gss [3]. Although quinoa grains have a high content of bioactive compounds, different agronomic and climatic conditions can affect the yields in their production [4].

Quinoa is a crop that can adapt to adverse climates (e.g., drought, frost, wind, hail, soil salinity, and soils with low fertility) [5–7]. This crop's yield depends on the periodicity and volume of rainfall (from 500 to 1200 mm) in the high Andean regions (from 2300 to 3800 m.a.s.l) [2], while in coastal areas (<1200 m.a.s.l) where there is no rainfall, irrigation is carried out by gravity and sprinklers from various surface water sources (rivers and lakes) [8]. However, surface waters could present critical concentrations of antibiotic residues, which can not only affect the production yield of the quinoa crop but also represent a risk to the consumer's health [9].

Antibiotics present in surface water result from their excessive use in agriculture and medicine, which can reach rivers and underground water [10]. Although different antibiotics are present in surface waters, CIP has the highest concentrations due to its use in humans and veterinary situations [11,12]. Unlike other antibiotics, this compound is more persistent in the environment and bioaccumulated by plants [9]. Although permissible limits for CIP in soils and surface waters have not been officially established, several studies have reported concentrations ranging from 0.37 to 0.40 mg/kg for soils and between 2.5 and 6.3 mg/L for surface water sources [13–15]. Thus, the use of water contaminated with CIP could affect plant growth and development of the quinoa [16,17].

CIP can potentially modify the processes of photosynthesis and respiration, leading to significant alterations in the physical characteristics of plants, including the root, stem, and leaf structures [11,18]. This compound induces toxic effects and hormesis in plants by generating hydrogen peroxide (H_2O_2), which triggers oxidative stress. Consequently, oxidative stress leads to decreased biomass and eventual cell death [12]. However, oxidative stress promotes the formation of ROS (reactive oxygen species); it can increase the production of secondary metabolites in the plant, a defense mechanism against oxidative damage [19,20].

Polyphenols are secondary metabolites and can be divided into different families, such as phenolic compounds, flavonols, and stilbenes, which present particular bioactive properties that can help prevent diseases related to oxidative stress [21]. These compounds are biosynthesized in the cytoplasm via the shikimic acid pathway from primary metabolites (amino acids and carbohydrates) [22–24]. In general, when plants encounter environmental stressors or are exposed to toxic compounds, reactive oxygen species (ROS) are generated. To counteract the damaging effects of these radicals, plants synthesize and accumulate polyphenols within their tissues [22,25]. Thus, these compounds act as powerful antioxidants, effectively scavenging the ROS and preventing them from causing oxidative damage to the plant's cells and tissues [26]. Although quinoa grain can present a high content of total polyphenols, between 39 and 198 mg GAE/100 gdw, the concentration of these compounds can vary due to external factors such as environmental and cultivation conditions, as well as due to the presence of CIP in the irrigation water.

In this sense, quinoa being a grain of nutritional importance, it is necessary to evaluate the effects of antibiotics on its growth and the production of polyphenols. Thus, we proposed assessing the impact of the CIP on morphological characteristics (root length, stem length, and panicle length) of the Pasankalla variety of quinoa, as well as analyzing the phenol content to demonstrate the impact of CIP on these metabolites.

2. Materials and Methods

2.1. Study Area

The location of the experiment was in Juliaca city, province of San Roman, Peru. The installation of the study was carried out in a greenhouse specially prepared for the conduction and controlled management of environmental conditions that did not interfere with the purpose of the research (15°30′47.2″ S, 70°07′40.3″ W). The quinoa variety used was Pasankalla. The antibiotic used was CIP since there is evidence of residues in the same surface waters in different regions of Peru [27,28].

2.2. Experimental Design of Exposure of Quinoa to Ciprofloxacin

The seeds were surface sterilized in a 2.5% sodium hypochlorite solution for 5 min [18], rinsed in distilled water, and planted in 20 L pots containing agricultural soil substrate with organic nutrients (Table 1); each pot received four seeds. Plants were grown under greenhouse conditions, initially watered twice a week with 500 mL of distilled water; 15 days after seedling emergence, the largest plant in each pot was selected, keeping only one plant/pot [18]. Next, the CIP stock solution was prepared in ultrapure water using analytical grade CIP, which was then used to prepare solutions with the desired test concentrations. Plants were exposed to concentrations of 0 (distilled/control water) and 1, 10, and 100 µg/L of CIP, based on a range of occurrences of this antibiotic in surface water.

Table 1. The physicochemical and mechanical parameters of experimental soil.

Soil Physicochemical Parameters			Mechanical Analysis		
Parameter	**Unity**	**Value**	**Parameter**	**Unity**	**Value**
pH	pH	7.7	Sand	%	33.6
Electrical conductivity	mS/m	138.7	Silt	%	35.3
Organic matter	%	2.0	Clay	%	31.1
Nitrogen	%	0.1	Textural class	-	Clay loam
Phosphorus	ppm	8.8			
Potassium	ppm	909.8			
Calcium carbonates	%	1.1			

Irrigation of the plants was performed twice a week with 500 mL of distilled water or experimental solutions containing CIP to maintain a field capacity of 70% of the substrate; irrigation was performed with a graduated cylinder at soil level. Twelve pots were conducted, with four treatments 1, 10, and 100 µg/L of CIP, respectively. In addition, a control was used to compare the effect of CIP.

2.3. Morphological Characteristics Analysis

In evaluating the height and weight of dry aerial biomass, dry weight of the panicle, root length, and dry root biomass weight, we used the recommended by Gomes et al. [18]. At the physiological maturity stage (117 days after sowing), the plants/treatments were harvested and divided into roots, leaves, and panicles using plastic trays to place the aerial part of the plant, washed three times in distilled water (500 mL/each), divided into leaves and stems, subsequently dried at 45 °C for 48 h, and then weighed. As for root length and dry root biomass weight, plastic trays were used, where the plants were carefully separated from the substrates (soil), washed three times in distilled water (500 mL/each), measured for their root length, dried at 45 °C, and finally, weighed. To weigh the panicle, plastic containers were also used to place each panicle, then the size of the panicle/plant was determined. The weight of the panicle was determined, they were dried at 45 °C, and finally, they were weighed again on an analytical balance.

2.4. Total Polyphenol Content (TPC)

The Total Phenolic Content (TPC) was determined following the method proposed by Singleton and Rossi [29]. In brief, the sample (0.5 mL) was mixed with distilled water (3.75 mL) and Folin–Ciocalteu reagent (0.25 mL). After, it was mixed with Na_2CO_3 (0.5 mL). The reaction mixture was then kept in the dark for one hour, followed by measuring the absorbance at 765 nm. Finally, TPC was quantified as gallic acid equivalent (GAE) per gram of dry weight, utilizing a standard curve of gallic acid ranging from 10 mg/L to 90 mg/L, with a high correlation coefficient (r2) of 0.9985.

2.5. Antioxidant Capacity by DPPH

The antioxidant capacity of the extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method [30]. Briefly, 0.1 mL of extract was mixed with 3.9 mL of DPPH solution (0.1 mM). Then, this mixture was incubated at room temperature in the dark for 30 min. The reduction in DPPH was then measured at 517 nm using a UV spectrometer (UV 1240, Shimadzu, Kyoto, Japan). The IC_{50} (mg/mL) represents the extract concentration required to inhibit 50% DPPH radical absorption.

2.6. Antioxidant Capacity by Oxygen Radical Absorbance Capacity (ORAC)

The extracts' antioxidant activity was determined according to the methodology proposed by Chirinos et al. [31]. The ORAC analyses were performed in a 96-well microplate fluorometer (Ascent F.L. Fluoroscan, Labsystem, Finland). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) (153 mM) was used as a peroxyl radical generator. Trolox (0.01 M) was used as the standard, and fluorescein (55 mM) was used as a fluorescent probe. Approximately 25 µL of phosphate buffer (75 mM) at pH 7.4 was used as the blank. After this, the Trolox standard or the diluted sample in phosphate-buffered saline (PBS) buffer at pH 7.4 were mixed with 250 µL of fluorescein and incubated for 10 min at 37 °C. An automatic 25 µL AAPH solution (153 mM) injection was added to all the microplates. The fluorescence was measured every minute for 50 min. The final ORAC values were calculated using the area under the curves and were expressed as µmol of Trolox equivalents per gram of dry weight (µmol TE/g dw).

2.7. Quantification of Target Polyphenols

Specific polyphenols were quantified according to the methodology of Maldonado et al. [32] with some modifications. A total of 100 µL of samples were diluted with methanol and filtered through a 0.22 mm membrane. Then, 2 µL of the filtered sample was injected into an ultra-performance liquid chromatography (Agilent 1290 Infinity II, Agilent, Santa Clara, USA) equipped with a diode array detector and reverse phase Poroshell C18 column (2.1 µm × 150 mm × 1.9 µm) at 30 °C. Chromatographic separation was carried out using a mobile phase consisting of A (acetonitrile and formic acid 0.1%) and B (water and formic acid 0.1%) in a gradient elution analysis programmed as follows: 95% A–5% B for 15 min, then 60% A–40% B for 18 min, and 95% A–5% B was maintained for 20 min, at a flow rate of 0.3 mL/min. Calibration curves were obtained by plotting peak areas versus different concentrations of standard solutions. Analyses were performed in triplicate, and results were expressed in µg of the specific polyphenol.

2.8. Statistics Analysis

The ANOVA test was performed with the response variables; after checking the assumptions of normality using the Shapiro–Wilk test and homogeneity using Bartlett's test. In the case of significant variables ($p < 0.05$), the means were compared using a Dunnet post hoc test (with a significance level of $p < 0.05$) for all comparisons. The analysis was made in R Studio version 4.2.1.

3. Results

The results were organized into two subsections, delineating the effects of antibiotic dosage on the physical characteristics of the plant as well as the content of bioactive compounds. It should concisely and precisely describe the experimental results, their interpretation, discussion, and conclusions.

3.1. Impact of Dosage of CIP on Some Physical Characteristics

3.1.1. Root Length and Plant Growth

The root length was not affected by the dosage of the antibiotic (CIP) (Table 2). Contrarily, the use of CIP affected plant growth ($p < 0.05$). For example, the use of low concentrations of CIP (1 µg/L) reduced its growth by 7% compared to the control, while

the plant growth decreased by 16% when a higher concentration of CIP (10 µg/L) was employed (Table 2).

Table 2. Length of some parts of the plant, according to the different treatments.

Treatments	Plant		Root	
	Mean	SD	Mean	SD
Control	50.50 b	±3.04	19.00 a	±2.65
1 µg/L	47.17 a,b	±1.61	19.67 a	±4.04
10 µg/L	40.17 a	±6.21	19.33 a	±1.53
100 µg/L	42.33 a	±2.31	20.83 a	±1.76

Control represents 0 µg/L. Mean is expressed as cm (n = 3) and SD: standard deviation (n = 3); for panicle and root, there are no differences between treatments ($p > 0.05$). Different lowercase letters in the same row indicate differences between treatments ($p < 0.05$) for each response variable.

3.1.2. Biomass of Some Parts of the Plant

According to our results, the biomass of some parts of the plant, such as the grain, panicle, stem, and root, was not affected by the dosage of CIP during physiological maturity (Table 3).

Table 3. Biomass of some parts of the plant, according to the different treatments.

Treatments CIP (µg/L)	Grain		Panicle		Stem		Root	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	0.81	±0.09	4.59	±1.03	12.61	±2.62	1.47	±0.21
1 µg/L	1.50	±0.63	4.08	±0.73	11.33	±1.08	1.24	±0.23
10 µg/L	0.79	±0.33	3.29	±0.95	10.32	±2.91	1.02	±0.28
100 µg/L	0.63	±0.04	2.87	±0.58	12.11	±0.84	1.54	±0.23

Control represents 0 µg/L. Mean is expressed as gram (n = 3) and SD: standard deviation (n = 3). For this analysis, there are no differences between treatments ($p > 0.05$).

3.2. Impact of Dosage of CIP on Antioxidant Compounds

3.2.1. Total Polyphenol Content (TPC)

The TPC value increased as the dosage of CIP increased. For example, the TPC increased ~23% and ~31% with 10 and 100 µg/L of CIP, respectively (Table 4).

Table 4. Chemical characterization of antioxidant compounds.

Treatments CIP (µg/L)	TPC (mg GAE/gdw)		DPPH (IC_{50}: mg/mL)		ORAC (µmol ET/gdw)	
	Mean	SD	Mean	SD	Mean	SD
Control	4.40 ^{b}	±0.06	8.37 ^{b}	±0.46	87.92 ^{b}	±6.08
1 µg/L	3.63 ^{a}	±0.05	10.28 ^{c}	±0.06	70.67 ^{a}	±5.44
10 µg/L	5.44 ^{c}	±0.09	5.71 ^{a}	±0.10	92.57 ^{b}	±4.05
100 µg/L	5.79 ^{d}	±0.13	5.42 ^{a}	±0.02	121.73 ^{c}	±5.62

Control represents 0 µg/L. TPC: Total Polyphenol Content was expressed as mg of gallic acid equivalent per gram of dry weight. IC_{50} was expressed as mg of sample to inhibit 50% of the DPPH radical solution (mL). ORAC was expressed as µmol Trolox equivalent per gram of dry weight. Different letters indicate statistically significant differences ($p < 0.05$).

3.2.2. Antioxidant Capacity

Polyphenols can be evaluated for their capacity to inhibit specific radicals using the DPPH and ORAC methods. The DPPH method measures the ability of polyphenols to neutralize DPPH radicals, while the ORAC method assesses the capacity of polyphenols to neutralize peroxyl radicals. For this study, as the CIP dose increased, the amount of sample required to inhibit the DPPH radical decreased. For example, the use of 10 µg/L of CIP

reduced by 32% of the sample needed to scavenge DPPH compared to the control (Table 4). On the contrary, the use of 10 µg/L of CIP exhibited the highest ORAC values (10 µg/L: 92.57 µmolTE/gdw) (Table 4).

3.2.3. Polyphenols Profile

The contents of some target phenolic acids and flavanols were quantified to explain the effect of CIP on these compounds (Table 5). The analysis results revealed that when increasing the CIP dosage from 0 to 100 µg/L, the production of phenolic acid increased 1.6-fold (Table 5). Vanillic acid was the most abundant phenolic acid quantified, where the high concentrations of CIP (100 µg/L) were able to recover 0.33 µg/gdw of this compound (Table 5).

Table 5. Polyphenol profile present in quinoa grain.

Description	Treatments							
	Control		1 µg/L		10 µg/L		100 µg/L	
Phenolic acid (µg/gdw)	Mean	CV %	Mean	CV %	Mean	CV %	Mean	CV %
Caffeic	0.08 ^{a}	30.20	0.09 ^{a}	16.37	0.11 ^{a,b}	24.05	0.18 ^{b}	13.73
Vanillic	0.21 ^{a}	16.50	0.26 ^{a}	17.63	0.22 ^{a}	12.30	0.33 ^{b}	4.68
Subtotal	0.29		0.35		0.33		0.51	
Flavanols (µg/gdw)								
Catechin	0.47 ^{a}	11.33	0.50 ^{a}	8.93	0.49 ^{a}	6.61	0.71 ^{b}	4.23
Epicatechin	0.20 ^{a}	19.88	0.20 ^{a}	10.24	0.22 ^{a}	13.68	0.38 ^{b}	8.11
Procyanidin B2	0.30 ^{a}	20.14	0.41 ^{a,b}	5.04	0.45 ^{b}	8.35	0.73 ^{c}	7.00
Procyanidin A2	0.91 ^{a}	11.83	0.92 ^{a}	8.10	0.92 ^{a}	12.30	2.92 ^{b}	2.43
Subtotal	1.89		2.04		2.09		4.74	

Results are expressed as µg per gram dry weight. CV: coefficient variation (n = 3). Different letters indicate statistically significant differences ($p < 0.05$). Lowercase letters indicate differences between treatments.

On the other hand, the production of flavanols exhibited a similar behavior compared to phenolic acids, with a 2.3-fold increase when the CIP dosage increased from 0 to 100 µg/L (Table 5). The recovery of specific flavonols, such as catechin, epicatechin, procyanidin B2 and A2, and epigallocatechin, increased with high concentrations of CIP (100 µg/L) (Table 5). Under these conditions, the samples presented a high procyanidin A2 concentration (2.92 µg/gdw) compared to other specific flavanols (Table 5).

4. Discussion

4.1. Root Length

As for the development of root length, the different treatments with CIP do not show a significant level. This could be due to the other response mechanisms demonstrated by quinoa against this contaminant, such as vacuole compartmentalization, which may play an important role in tolerance and detoxification, thus preventing circulation in the cytosol and restricting the contaminant to a limited area [33,34]. In this sense, the phases leading to the detoxification of organic pollutants are transformation (Phase 1), conjugation (Phase 2), and compartmentalization (Phase 3) [34,35]. Enzymes like NADPH-cytochrome P450 reductase and glutathione transferase are essential in the control of inflammation and cytotoxicity; they are responsible for catalyzing the one-electron reduction in numerous drugs and foreign substances in plants [35]. Likewise, Zhao et al. [36] report that CIP toxicity-tolerant roots help maintain standard cell structure and function, accumulating this compound through continuous and long-term antibiotic absorption [16]. Since the half-life of CIP is 90 days, we can understand that it is not easily degraded and is persistent in the environment and plants [37]. In addition, it is mentioned that the detoxification of organic pollutants in plants can be driven by the metabolism performed by a series of enzymes inside plant cells [38]. In addition, the distribution of organic contaminants within the plant is related to water solubility and the octanol–water partition coefficient

(log Kow), where hydrophilic organic xenobiotics (log K_{ow} 1–4) are easily absorbed by roots and translocated [39].

Although hydrophobic organic xenobiotics are unlikely to be absorbed and transported by plants, CIP is strongly hydrophilic [40]. Thus, quinoa roots can absorb this compound, which is accumulated in the water [41]. However, it is known that quinoa roots grow even to 120 cm too long in some conditions [42], showing the resistant capacity of quinoa in front of adverse and polluted condition environments.

Although there was no statistically significant difference ($p > 0.05$) in root length between treatments, a slight elongation of the root was observed concerning the control, which may be associated with changes in the oxidative state of the plant organ, where treatments with CIP show increased levels of H_2O_2 in the roots, with benefits for their development [43] since the low concentration of antibiotics can induce hormesis by altering a wide range of plants' physiological, biochemical, cellular, production, and growth properties, ranging from the promotion of cell division to stimulation of biomass accumulation [44].

4.2. Plant Growth Retardation

Plant growth was reduced when high concentrations of CIP (100 µg/L) were used compared to the control; the decrease in plant growth may be related to CIP interfering with mitochondrial functions [43], organelles that are closely associated with the production of ATP and reducing power (NADH); thus, this compound interferes with the assimilation of nitrogen in the form of nitrate (NO_3^-) because energy expenditure is required. For example, it was shown that CIP decreases the ability to fix or assimilate nitrogen by 34 and 66%, respectively; in *Azolla-Anabaena*, the reduction in the fern of the process of nitrogen fixation may be secondary, i.e., as a result of its effects on energy metabolism [45]. Similarly, CIP interference with photosynthesis may decrease the production of carbon skeletons for cellular activities [46]. This affects the photosynthesis process; it has been observed that CIP induces intracellular ROS formation; in particular, H_2O_2 would result in lower assimilation of nitrogen and carbon and, consequently, in reduced plant growth [45,47]. Conversely, the use of antibiotics on the apical meristem has shown effects on the ability of cells to promote their development [48]. This could explain why, in our study, quinoa plant growth exposed to IPC was negatively affected.

4.3. Weight of Root and Stem Biomass

Our study revealed that quinoa plants could tolerate up to 100 μgL^{-1} of CIP in irrigation water; no significant reduction in root biomass dry weight and stem dry weight was observed. This may be due to the genes in its cellular structure that play a fundamental role that allows it to survive in hypersaline conditions, i.e., it is related to tolerance to organic and inorganic xenobiotics [49]; For example, Guarino et al. [50] concluded that quinoa subjected to stress by inorganic contaminants shows greater affectation in genes at the foliar level than in roots; however, there was no effect on dry root weight nor at the foliar level, which is in line with the results reported herein. Thus, this finding suggests greater responsiveness in leaves, where metabolic activities that are crucial for plant growth and productivity, namely photosynthesis, occur. Additionally, in the case of CIP, the log K_{ow} is 0.75 [51], meaning it is highly soluble in water; therefore, it can be absorbed and translocated in plant tissues. Subsequently, these organic contaminants can be stored, volatilized, or completely mineralized to produce carbon dioxide and water. Moreover, quinoa leaves contain phenolic compounds, such as ferulic, synaptic, gallic acid, kaempferol, and isorhamnetin [52]. This highlights the central role of phenolic compounds in protecting the plant from stress because they can scavenge free radicals produced in CIP-induced oxidative stress. Contrastingly, it has been suggested that polyphenols and other antioxidants have a high capacity in halophytic species compared to glycophytes [53]. Thus, halophytic species act as signaling agents in response to H_2O_2 stress and have an efficient antioxidant mechanism to buffer and scavenge reactive oxygen species (ROS). Consequently,

enzymes and the antioxidant defense system collaborate to provide plants with long-term resistance to oxidative stress. That collapse of either system would likely reduce the overall antioxidant capacity of the plant and cause cellular oxidative damage [46].

4.4. Grain and Panicle

It has been demonstrated that halophytic plants tend to degrade organic pollutants; for example, quinoa can partially degrade antibiotics in plant tissue; this is confirmed by the decrease in the concentration of sulfamethazine in the stem and the culture medium [54]; it is also known that sulfamethazine is not found in the grains, so the author suggested that this organic compound is transported within the plant through passive diffusion, without the need for energy input; however, translocation to seeds requires ATP and NADH energy, also known as active diffusion [54]. This could explain why CIP did not affect grain dry weight. On the other hand, it is probably due to blocking the transport of storage materials from stems to grain [55], which could be due to the presence of a bladder gland in quinoa leaves, and are believed to be storage sites for excess Na^+, Cl^-, and K^+, which are then expelled [55]. This could represent a positive finding as quinoa is grown mainly because of its grains [56]. However, these absences can cause grain yield decline, adversely affecting energy metabolism and mineral nutrition (Marques et al., 2021). On the other hand, although there is no significant difference in dry weight and panicle size between treatments, a slight decrease in the presence of CIP was observed in both treatments. This could be part of a plant stress response from antibiotics or other abiotic factors.

4.5. Total Polyphenol Content and Antioxidant Activity

According to our results, the total polyphenol content varies between 3.63 and 5.49 mg GAE/gdw. This value is higher than the other results reported by Li et al. [57] and Goméz-Caravaca et al. [58], with 2.79 and 3.84 mg GAE/gdw, respectively. Probably, when agronomic conditions like soil pH, nutrients, and irrigation period are controlled, the production of metabolites in quinoa grains is more efficient than in other studies.

Although ultraviolet radiation and the presence of pathogens induce the production of ROS in plants, the presence of antibiotics can also stimulate intracellular ROS production (i.e., mitochondria and plasma membrane) [59,60]. Thus, ROS production exceeding normal levels can cause irreversible damage to DNA, lipid peroxidation, and protein oxidation, ultimately leading to cell death [36]. Nonetheless, plants possess defense mechanisms involving the production of polyphenolic compounds, which mitigate ROS due to their potent antioxidant and reducing capacity [19]. The production of these compounds occurs through the shikimic acid pathway, which involves the conversion of phenylalanine and tryptophan amino acids to phenolic acids [61–63]. Thus, it is probable that in our study, CIP had the potential to induce stress conditions in quinoa grains, leading to a significant increase in the production of reactive oxygen species (ROS). However, as a defense response or protective mechanism, the grains also stimulate the production of polyphenols through the shikimic acid pathway, which has the ability to effectively reduce ROS levels.

On the other hand, polyphenols' ability to inhibit specific radicals can be determined using the DPPH and ORAC assays. The DPPH method measures polyphenols' capacity to neutralize DPPH. This free radical is distinct from other biological reactive species (peroxyl radicals), while the ORAC method assesses polyphenols' capacity to neutralize peroxyl radicals [64]. Thus, the higher the polyphenols' content in the grains, the greater their antioxidant capacity.

4.6. Polyphenolic Profile

The prevalence of vanillic acid as the principal compound within the phenolic acid in its free form follows the results showed by Antognoni et al. [65] and Tang et al. [66], who reported that this hydroxybenzoic acid is the primary compound in various ecotypes of quinoa grains, including red, white, and black [67]. In addition, the presence of CIP induces a greater production of vanillic acid compared to the control. In general, plant

cells primarily utilize ferulic acid as a precursor for the production of vanillin through the continuous catalytic action of vanillin synthase; this enzymatic process facilitates the direct synthesis of vanillic acid [62]. Thus, it is probable that the use of CIP promotes a higher enzymatic activity to produce vanillic acid in order to inhibit the presence of ROS. This opens the possibility of planning future strategies for the selective production of specific polyphenols with important bioactive properties.

Although the presence of flavanol monomers like catechin and epicatechin in quinoa has been reported by other studies [65–67], in this study, the presence of CIP not only induces a greater production of flavanol monomers, but also produces a high procyanidins content (Table 4). Procyanidins are polymers commonly known as flavan-3-ols, which are composed of catechin and epicatechin monomers, which are glycosylated with gallic acid as the terminal molecule [62,68].

The biosynthesis of these compounds is primarily regulated by the phenylpropanoid metabolic route [69]. This mechanism modulates the activity of transcription factors that bind to specific DNA sequences, which leads to gene expression in the polyphenol biosynthesis pathway [69–71]. These genes are recognized as the Expression of Early Biosynthetic Genes (EBG), which include phenylalanine ammonia lyase, cinnamate 4-hydroxylase, 4-coumarate coenzyme A ligase, chalcone synthase, and chalcone isomerase [72]. In particular, this mechanism can promote the production of leucoanthocyanidin reductase, an enzyme that catalyzes the synthesis of catechin and serves as the initial committed step in proanthocyanidin biosynthesis [73]. Thus, it is probable that CIP presence induces a higher enzyme activity, leading to a significant increase in the production of procyanidins.

5. Conclusions

The biomass, grain, and panicle of the Pasankalla variety of quinoa were affected when exposed to high concentrations of CIP, while other morphological parameters remained unaffected. These results suggest that this species is resistant to the doses used in the study. Contrarily, high CIP concentrations stimulated the biosynthesis of polyphenols with potent antioxidant properties, surpassing the levels observed in the control group. Notably, the production of specific polyphenols, such as vanillic acid, procyanidins A2, and procyanidins B2, was significantly enhanced when CIP was applied. Finally, although CIP residues in the grains would represent a risk to the population's health, under controlled conditions, CIP could be used as a stimulator of bioactive compounds.

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