EDINEI JOSÉ ARMANI BORGHI

ROLE OF SELENIUM IN STRESS MITIGATION AND ENHANCING CONILON COFFEE QUALITY: A STUDY OF SELENIUM SOURCES AND RATES

LAVRAS – MG
2025

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Advisor
Prof.ª DSc. Maria Ligia de Souza Silva

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EDINEI JOSÉ ARMANI BORGHI

PAPEL DO SELÊNIO NA MITIGAÇÃO DE ESTRESSES E NA MELHORIA DA QUALIDADE DO CAFÉ CONILON: UM ESTUDO DE FONTES E DOSES DE SELÊNIO

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LAVRAS – MG

2025

To my parents, Jair and Josefina.
To my sister, Camila
I dedicate!

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To God, who illuminated my path and supported me through the most challenging moments.

To my family — my father, Jair José Borghi, my mother, Josefina Armani Borghi, and my sister, Camila Armani Borghi — for their teachings, unwavering support, immeasurable love, and prayers.

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"When something is important enough, you do it even if the odds are not in your favor."

(Elon Musk)

RESUMO GERAL

O selênio (Se) desempenha um papel crucial no metabolismo antioxidante e reduz o estresse oxidativo sob condições de estresse abiótico (temperaturas extremas, seca, salinidade e metais pesados). Além disso, a biofortificação agronômica tem se destacado como uma estratégia eficaz para melhorar a qualidade nutricional dos alimentos e combater a fome oculta associada à deficiência de Se. O primeiro capítulo desta tese teve como objetivo avaliar a eficácia de diferentes fontes e doses de Se aplicadas via foliar na mitigação do estresse e na produção de café Conilon durante o fenômeno ENSO, por meio da avaliação de parâmetros nutricionais, fisiológicos e produtivos da cultura. O segundo capítulo teve como objetivo avaliar a biofortificação dos grãos de café Conilon por meio da aplicação foliar de diferentes fontes e doses de Se, e seus efeitos sobre os atributos físico-químicos dos grãos, a produção e a qualidade da bebida. Dois experimentos foram conduzidos simultaneamente em delineamento em blocos casualizados, em arranjo fatorial 2×5, com quatro repetições. O selenato de sódio (Na_2SeO_4 – Se(VI)) e o Se-bisglicinato (Se-Bis) foram aplicados via foliar em cinco doses de Se (0, 10, 20, 40 e 60 mg L^{-1}) durante o estádio de chumbinho. No primeiro estudo, a aplicação foliar de Se(VI) e Se-Bis aumentou significativamente os teores foliares de Se, sem causar toxicidade ou alterar os teores de macro e micronutrientes. O fornecimento de Se aumentou a concentração foliar de prolina e a atividade de enzimas antioxidantes [superóxido dismutase (SOD), ascorbato peroxidase (APX) e catalase (CAT)], reduziu a peroxidação lipídica e melhorou o desempenho fotossintético, promovendo o acúmulo de amido nas folhas. A aplicação de Se na dose de 20 mg L^{-1} resultou em ganhos de produtividade de 21,60% e 13,77% em relação ao controle. Além disso, os resultados sugerem que a aplicação foliar de Se estimula a conversão de aminoácidos livres totais (AA) em proteínas solúveis totais (PST) em plantas sob condições de estresse. No segundo estudo, a aplicação foliar de Se na dose de 60 mg L^{-1} melhorou a qualidade do café e aumentou a concentração de Se nos grãos crus e torrados, contribuindo com 3,11%–4,52% (3–4 xícaras por dia) da ingestão diária recomendada para adultos. A aplicação de Se até 60 mg L^{-1}, utilizando Se(VI) e Se-Bis, biofortificou o café de forma eficaz, sem induzir sintomas visuais de toxicidade. Por outro lado, perdas significativas de Se ocorreram durante a torra (38,92%–48,00%) e o preparo da bebida (98,54%–99,00%). Esses resultados indicam que a aplicação foliar de Se(VI) e Se-Bis na dose de 20 mg L^{-1} de Se durante o estádio de chumbinho mostrou-se uma abordagem prática para reduzir o estresse oxidativo, melhorar o desempenho fotossintético e garantir maior produtividade do café Conilon durante o fenômeno ENSO. Além disso, a aplicação foliar de Se melhora a qualidade do café tanto nos aspectos sensoriais quanto nutricionais, oferecendo uma estratégia promissora para aumentar a ingestão dietética de Se.

Palavras-chave: *Coffea canephora*; mudanças climáticas; estresses abióticos; análise sensorial; segurança alimentar.

GENERAL ABSTRACT

Selenium (Se) plays a crucial role in antioxidant metabolism and reduces oxidative stress under abiotic stress conditions (*e.g.*, extreme temperatures, drought, salinity, and heavy metals). Furthermore, agronomic biofortification has emerged as an effective strategy to enhance the nutritional quality of food and address hidden hunger related to Se deficiency. The first chapter of this thesis aimed to evaluate the effectiveness of different sources and rates of foliar-applied Se in mitigating stress and Conilon coffee production during the ENSO phenomenon by measuring the crop's nutritional, physiological, and productive parameters. The second chapter aimed to assess the biofortification of Conilon coffee beans by testing the foliar application of different Se sources and rates and assessing their effects on the physicochemical attributes of the beans, yield, and beverage quality. Two experiments were conducted simultaneously using a randomized block design with a 2×5 factorial arrangement and four replications. Sodium selenate (Na_2SeO_4 – Se(VI)) and Se-bisglycinate (Se-Bis) were foliar-applied at five Se rates (0, 10, 20, 40, and 60 mg L^{-1}) during the small green fruit stage. In the first study, foliar application of Se(VI) and Se-Bis significantly increased foliar Se contents without causing toxicity or altering macronutrient and micronutrient contents. Selenium supply increased foliar proline concentration and antioxidant enzyme activity [superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT)], reduced lipid peroxidation, and improved photosynthetic performance, promoting starch accumulation in the leaves. Selenium applied at 20 mg L^{-1} resulted in yield gains of 21.60% and 13.77% compared with the control. Furthermore, the findings suggest that foliar application of Se stimulates the conversion of total free amino acids (AA) into total soluble proteins (TSP) in plants under stress conditions. In the second study, foliar Se application at 60 mg L^{-1} enhanced coffee quality and increased Se concentration in green and roasted beans, contributing to 3.11%–4.52% (3–4 cups day^{-1}) of the recommended daily intake for adults. Selenium application up to 60 mg L^{-1}, using Se(VI) and Se-Bis, effectively biofortified coffee without inducing visual toxicity symptoms. On the other hand, significant Se losses occurred during roasting (38.92%–48.00%) and brewing (98.54%–99.00%). These findings indicate that the foliar application of Se(VI) and Se-Bis at 20 mg L^{-1} of Se during the small green fruit stage proved to be a practical approach for reducing oxidative stress, enhancing photosynthetic performance, and ensuring higher yield of Conilon coffee during the ENSO phenomenon. Furthermore, foliar Se application enhances coffee quality in both sensory and nutritional aspects, offering a promising strategy for increasing Se intake in the diet.

Keywords: *Coffea canephora*; climate change; abiotic stresses; sensory analysis; food security.

INDICADORES DE IMPACTO

As descobertas deste estudo se enquadram em três das oito áreas temáticas da Política Nacional de Extensão do Brasil: Tecnologia e Produção, Meio Ambiente e Saúde. Os resultados destacam potenciais impactos tecnológicos, econômicos, sociais e ambientais da aplicação foliar de selênio (Se), especificamente nas formas de selenato de sódio (Na_2SeO_4) e Se-bisglicinato, no cafeeiro Conilon cultivado sob condições climáticas adversas, como altas temperaturas. A aplicação foliar de Se, especialmente na dose de 20 mg L^{-1}, resultou em um aumento de até 21,60% na produtividade do café Conilon durante o fenômeno ENSO. Isso representa um avanço tecnológico no manejo da cultura e mostra potencial para benefícios econômicos diretos em regiões produtoras como o Espírito Santo – maior produtor de café Conilon no Brasil. Além disso, a biofortificação do café forneceu até 4,52% da ingestão diária recomendada de Se para adultos, sugerindo um possível benefício à saúde pública por meio do aumento da ingestão dietética desse nutriente. É importante destacar que os resultados estão alinhados com os Objetivos de Desenvolvimento Sustentável (ODS) das Nações Unidas, especialmente o ODS 2 (Fome Zero e Agricultura Sustentável), ODS 3 (Saúde e Bem-Estar), ODS 12 (Consumo e Produção Responsáveis) e ODS 13 (Ação Contra a Mudança Global do Clima). Este estudo apresenta uma estratégia promissora para aumentar a resiliência da cafeicultura frente às mudanças climáticas, melhorar a qualidade do café, tanto sensorial quanto nutricionalmente, e gerar benefícios econômicos e sociais ao longo da cadeia de valor do café.

IMPACT INDICATORS

The findings of this study fall within three of the eight thematic areas of Brazil's National Extension Policy: Technology and Production, Environment, and Health. The results highlight the potential technological, economic, social, and environmental impacts of foliar selenium (Se) application, specifically in the forms of sodium selenate (Na_2SeO_4) and Se-bisglycinate, on Conilon coffee plants grown under adverse climatic conditions, such as high temperatures. Applying Se foliar spray, particularly at a concentration of 20 mg L^{-1}, led to an increase in Conilon coffee yield by up to 21.60% during the ENSO phenomenon. This represents a technological advancement in crop management and shows promise for direct economic benefits in production regions like Espírito Santo – the largest Conilon coffee-producing state in Brazil. Additionally, coffee biofortification provided up to 4.52% of the recommended daily intake of Se for adults, suggesting a potential public health benefit through increased dietary intake of this nutrient. It is important to note that the results align with the United Nations Sustainable Development Goals (SDGs), particularly SDG 2 (Zero Hunger and Sustainable Agriculture), SDG 3 (Good Health and Well-Being), SDG 12 (Responsible Consumption and Production), and SDG 13 (Climate Action). This study presents a promising strategy to enhance the resilience of coffee farming in the face of climate change, improve coffee quality, both sensorially and nutritionally, and generate economic and social benefits throughout the coffee value chain.

SUMMARY

FIRST PART

1 GENERAL INTRODUCTION

The United Nations (UN) projects that the global population will reach 8.5 billion by 2030, 9.7 billion by 2050, and 10.4 billion by the 2080s (UN, 2024). To meet the expected food demand resulting from a population of 9.7 billion in 2050, global food production must increase by 70% (FAO, 2024). Food security is a humanitarian concern and is part of the United Nations' Sustainable Development Goals to be achieved by 2030: “End hunger, achieve food security and improved nutrition and promote sustainable agriculture” (UN, 2015). Achieving food security through sustainable practices is essential for building long-term resilience in food systems, ensuring that communities can withstand environmental, economic, and social challenges while providing reliable access to nutritious food for future generations (TENDALL *et al*., 2015).

Coffee, one of the world's most traded commodities, is produced in approximately 50 countries, playing a significant role in job creation and socioeconomic development (BOUDREAU; CAJAL-GROSSI; MACCHIAVELLO, 2023). Among the 125 species of the *Coffea* genus, only two account for approximately 99% of the global commercial coffee production: *Coffea arabica* L. (Arabica coffee) and *C. canephora* Pierre ex A. Froehner (Conilon coffee) (DAMATTA *et al*., 2018; DAVIS *et al*., 2011). According to the United States Department of Agriculture (USDA, 2024), Brazil is the world's largest coffee producer, followed by Vietnam, Colombia, and Indonesia, and the second-largest coffee consumer, surpassed only by the United States. According to the National Supply Company (CONAB, 2023), Brazil produced 50.92 million 60-kilogram bags of coffee in 2022, of which 64.25% was Arabica coffee and 35.75% was Conilon coffee. Of this total, 39.8 million bags were exported, with the United States, Germany, Italy, Belgium, and Japan as the main destinations.

Global coffee consumption has been increasing at an average annual growth rate of approximately 2% (VEGRO; ALMEIDA, 2020). In China, coffee consumption has grown significantly, increasing nearly 150% in the last 10 years (USDA, 2024). Conversely, adverse climatic conditions have significantly affected the coffee supply, raising concerns about climate change (PHAM *et al*., 2019; TAVARES *et al*., 2018). Drought and unfavorable temperatures are the main climatic factors that induce abiotic stress in coffee plants and limit crop yield (BORGO *et al.*, 2025; DAMATTA; RAMALHO, 2006). In Brazil, these adverse conditions are intensified by the El Niño Southern Oscillation (ENSO) phenomenon, which decreases precipitation, increases air temperature, and exacerbates water deficits in major coffee-producing regions (SILVA *et al.*, 2020a).

Abiotic stresses significantly disrupt plant physiology at molecular, biochemical, and morphological levels, often causing irreversible damage (BORGO *et al.*, 2025). Under stress conditions, there is an overproduction of reactive oxygen species (ROS), such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH\cdot$), singlet oxygen ($^1O_2$), methyl radical ($CH_3\cdot$), and lipid peroxidation free radicals ($LOO\cdot$ and $ROO\cdot$) (FENG; WEI; TU, 2013). ROS impair cellular redox homeostasis, causing oxidative damage to biomolecules (*e.g.*, lipids, proteins, DNA, and carbohydrates), which can be irreversible and lead to cell death, compromising crop development and yield (HASANUZZAMAN *et al.*, 2020a; LANZA; REIS, 2021). Moreover, adverse climatic conditions can influence the chemical composition of coffee beans, affecting beverage quality and its commercial value (AGNOLETTI *et al.*, 2022; SIMMER *et al.*, 2022).

Selenium (Se) is essential for humans and animals, and it is considered a beneficial element for plants (CHAUHAN *et al.*, 2019; RENGEL; CAKMAK; WHITE, 2022). In plants, Se acts in antioxidant metabolism, reducing oxidative stress caused by ROS and conferring tolerance to various abiotic stresses (*e.g.*, extreme temperatures, drought, salinity, and heavy metals) (FENG; WEI; TU, 2013; RENGEL; CAKMAK; WHITE, 2022). Selenium is involved in the formation of selenoproteins, which act as powerful antioxidants in plant metabolism through the glutathione peroxidase (GSH) pathway, and by increasing the activity of enzymatic compounds (*e.g.*, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR)) and non-enzymatic compounds (*e.g.*, carotenoids, flavonoids, ascorbic acid, and tocopherols), which act in the ROS elimination system (LANZA; REIS, 2021).

The benefits of Se application have been observed in various crops, including soybean (*Glycine max* (L.) Merr.) (SILVA *et al.*, 2024), sugarcane (*Saccharum officinarum* L.) (ARAUJO *et al.*, 2023), and cowpea (*Vigna unguiculata* (L.) Walp.) (SILVA *et al.*, 2020b). In Arabica coffee, foliar application of Se reduced oxidative stress, increased the activity of antioxidant enzymes (SOD, APX, CAT, and GR), enhanced the levels of photosynthetic pigments (chlorophylls, pheophytins, and carotenoids), and promoted yield increases of 38% and 42% compared to control (MATEUS *et al.*, 2021). For Conilon coffee, foliar Se application reduced cold stress-induced leaf damage by 24% in seedlings grown under controlled conditions (SOUSA *et al*., 2022). Furthermore, recent studies have shown that early foliar Se application, performed 8 days before stress induction, significantly reduced oxidative stress in Arabica coffee seedlings (SOUSA *et al*., 2023). The combined effects of biosynthesis of photoprotective pigments and the enhancement of antioxidant metabolism induced by Se are the main physiological mechanisms in the photochemistry of photosynthesis, promoting increased photosynthetic performance in various plant species (HASANUZZAMAN *et al*., 2020b; LANZA; REIS, 2021). Thus, proper Se application can promote plant growth and development by stimulating photosynthesis and favoring starch and sugar accumulation (YANG *et al*., 2022).

The range between the optimal and toxic levels of Se is narrow and varies among species (BROWN; SHRIFT, 1982; WHITE, 2018). The Se content in plants is directly related to its content and availability in soils (PRADO; CRUZ; FERREIRA, 2017). Most agricultural soils in various regions of the world exhibit low Se availability (LOPES; ÁVILA; GUILHERME, 2017). In Brazil, Se levels in soils range from 10 to 650 μg kg^{-1} (REIS *et al*., 2017). In the Cerrado soils, Se levels show less variation, with values ranging from 22 to 72 μg kg^{-1} (CARVALHO *et al*., 2019).

The availability of Se is not exclusively related to its total content in the soil but varies depending on soil attributes and the predominant chemical forms of Se (LOPES; ÁVILA; GUILHERME, 2017). Selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) are the main inorganic forms of Se found in the soil (HASANUZZAMAN *et al*., 2020b; WHITE, 2016). The adsorption of Se, particularly in the form of selenite, is significantly affected by the physicochemical properties of the soil, such as pH, Eh, clay content, organic matter, metal oxides, and competitive anions (PO_4^{3-}, SO_4^{2-}, and NO_3^{-}) (DINH *et al*., 2019). In tropical soils, selenate adsorption occurs preferentially through outer-sphere complexation, while selenite is predominantly adsorbed through inner-sphere complexation (specific adsorption), exhibiting lower mobility and availability to plants (ARAUJO *et al*., 2018, 2020).

The availability of Se in the soil directly influences its content in food and, consequently, the amount of Se consumed by the population (LOPES; ÁVILA; GUILHERME, 2017; PRADO; CRUZ; FERREIRA, 2017). The recommended daily intake of Se for adults is 70 μg day^{-1} (KIPP *et al*., 2015). However, Se deficiency in the human diet is a global issue, affecting approximately 15% of the world's population (HAWRYLAK-NOWAK, 2013; SCHIAVON *et al*., 2020). Low dietary Se intake is associated with increased mortality, heightened viral virulence, impaired immune function, Keshan disease, Kashin-Beck disease, autoimmune thyroid disease, fertility/reproductive issues, cognitive decline, dementia, type 2 diabetes, prostate cancer risk, and colorectal cancer risk in women (RAYMAN, 2020). Furthermore, total serum Se concentrations were significantly lower in non-surviving COVID-19 patients (MOGHADDAM *et al*., 2020).

Some approaches are proposed to promote adequate nutrient intake and combat human malnutrition, including dietary diversification, supplementation, fortification, and genetic and agronomic biofortification (RAWAT *et al*., 2013; SCHIAVON *et al*., 2020). Biofortification aims to increase the nutritional value of foods and is considered a feasible and more economical approach to overcoming hidden hunger (CAKMAK, 2008; GUPTA; GUPTA, 2017; YUAN *et al*., 2012). Agronomic biofortification aims to enhance the nutritional value of food through the optimized application of fertilizers (CAKMAK, 2008; CAKMAK; KUTMAN, 2018). Several studies on agronomic biofortification have shown a significant increase in the Se content and accumulation in the edible parts of various crops, such as rice (BOLDRIN *et al*., 2013), lettuce (RAMOS *et al*., 2011), tomato (SCHIAVON *et al*., 2013), and wheat (GALINHA *et al*., 2014). Agronomic biofortification with Se can improve agricultural products' nutritional and biochemical quality, increase crop productivity, and provide various health benefits (LANZA; REIS, 2021).

Agronomic biofortification studies should focus on foods or beverages widely consumed by the population (BOLDRIN *et al*., 2013; MATEUS *et al*., 2021) Coffee is one of the most consumed beverages globally, with increasing demand in nearly every region of the world (VEGRO; ALMEIDA, 2020). The impacts of coffee consumption on human health have been extensively studied and shown to be associated with a reduction in mortality rates from various causes, such as diabetes mellitus, dementia, Parkinson's disease, cardiovascular diseases, and cancers (BARREA *et al*., 2021; SAFE *et al*., 2023). These effects are related to the complex chemical composition of coffee, which contains more than a thousand bioactive compounds, such as caffeine, trigonelline, phenolic compounds, diterpenes, and soluble fibers (BARREA *et al*., 2021). Therefore, agronomic biofortification of coffee is a promising approach to increasing Se dietary intake and enhancing the beverage's health benefits.

Selenium appears to be a promising strategy for coffee cultivation to reduce abiotic stresses and enhance the intake of this element in the human diet. However, the existing literature lacks information on the effects of Se application—including the sources and rates—on Conilon coffee grown in field conditions, particularly concerning yield, biofortification, and quality parameters. Plant nutrition as a tool for plant stress mitigation and biofortification must follow the core concept of 4R Nutrient Stewardship (applying the Right Source, at the Right Rate, at the Right Time, and in the Right Place) (BORGO *et al*., 2025;

JOHNSTON; BRUULSEMA, 2014). In general, most studies have focused on the use of inorganic Se sources with rapid release, such as sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4) (LANZA; REIS, 2021; SCHIAVON *et al*., 2020; SILVA *et al*., 2020b). However, recent studies have demonstrated the great potential of using organic Se sources (Se-Glycine), which release more slowly and present a lower risk of causing plant toxicity (LI *et al*., 2023).

This thesis is divided into two chapters. The first chapter, titled “Foliar selenium application mitigates stress induced by El Niño-Southern Oscillation (ENSO) and enhances Conilon coffee yield”, aimed to evaluate the nutritional, physiological, and productive responses of Conilon coffee to foliar application of different sources and rates of Se at the small green fruit stage during the El Niño Southern Oscillation (ENSO) phenomenon. The second chapter, titled “Agronomic biofortification with selenium enhances Conilon coffee quality and contributes to human selenium intake: a study of selenium sources and rates” aimed to evaluate the biofortification of Conilon coffee through the foliar application of different sources and rates of Se and their effects on the physical-chemical attributes of the beans and beverage quality. The initial hypotheses of this thesis were: i) foliar application of sodium selenate and Se-bisglycinate in appropriate Se rates reduces oxidative stress, ensuring higher yield of Conilon coffee during the ENSO phenomenon; ii) foliar application of sodium selenate and Se-bisglycinate in appropriate Se rates promotes grain biofortification without affecting beverage quality.

SECOND PART

Article 1 – Written following the scientific journal's guidelines – Submitted version	
Article title:	Foliar Selenium Application Mitigates Stress Induced by El Niño-Southern Oscillation (ENSO) and Enhances Conilon Coffee Yield
Authors:	Edinei José Armani Borghi Leônidas Canuto dos Santos Fabrício Teixeira de Lima Gomes Amanda Santana Chales Francis Eliakin Portillo Magaña Maria Ligia de Souza Silva André Rodrigues dos Reis Flávio Henrique Silveira Rabêlo Plinio Rodrigues dos Santos Filho Marcone Comério Luiz Roberto Guimarães Guilherme
Journal:	Plant and Soil
ISSN	1573-5036

Article 1 – Foliar Selenium Application Mitigates Stress Induced by El Niño-Southern Oscillation (ENSO) and Enhances Conilon Coffee Yield

Abstract

Background and aims: Selenium (Se) acts in antioxidant metabolism and reduces oxidative stress under abiotic stress conditions. This study aimed to evaluate the effectiveness of different sources and rates of foliar-applied Se in mitigating stress and Conilon coffee production during the ENSO phenomenon by measuring the crop's nutritional, physiological, and productive parameters.

Methods: Two experiments were conducted simultaneously in a randomized block design, with a 2×5 factorial scheme and four replications. Two Se sources (sodium selenate: Se(VI) and Se-bisglycinate: Se-Bis) were applied via foliar sprays during the small green fruit stage at five Se rates (0, 10, 20, 40, and 60 mg L^{-1}).

Results: Foliar application of Se(VI) and Se-Bis significantly increased foliar Se contents without causing toxicity or altering macronutrient and micronutrient contents. Selenium supply increased foliar proline concentration and antioxidant enzyme activity [superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT)], reduced lipid peroxidation, and improved photosynthetic performance, promoting starch accumulation in the leaves. Selenium applied at 20 mg L^{-1} resulted in yield gains of 21.60% and 13.77% compared with the control. Furthermore, our findings suggest that foliar application of Se stimulates the conversion of total free amino acids (AA) into total soluble proteins (TSP) in plants under stress conditions.

Conclusions: Foliar application of Se(VI) and Se-Bis at 20 mg L^{-1} of Se during the small green fruit stage proved to be a practical approach for reducing oxidative stress, enhancing photosynthetic performance, and ensuring higher yield of Conilon coffee during ENSO and in the context of climate change.

Keywords *Coffea canephora*. Climate change. Abiotic stress. Antioxidant metabolism. Photosynthesis.

1 Introduction

Coffee is produced in approximately 50 countries within the "coffee belt," between 25° North and South of the Equator, and represents the primary source of livelihood for about 25 million smallholder farmers (Boudreau et al. 2023). Two main coffee species are produced in these regions, which account for approximately 99% of global commercial coffee production: *Coffea arabica* L. (Arabica coffee) and *C. canephora* Pierre ex A. Froehner (Conilon coffee) (DaMatta et al. 2018). Both species are susceptible to progressive climate change (DaMatta et al. 2019; Pham et al. 2019). Drought and extreme temperatures (cold and heat) are the main climatic limitations that induce abiotic stress in coffee plants and reduce crop yield (DaMatta and Ramalho 2006; Borgo et al. 2025). In Brazil, the world's largest coffee producer, these adverse conditions are intensified by the occurrence of the El Niño Southern Oscillation (ENSO) phenomenon that decreases precipitation, increases air temperature, and exacerbates water deficits in major coffee-producing regions (Silva et al. 2020a).

ENSO refers to variations in atmospheric and oceanic conditions resulting from changes in sea surface temperatures and atmospheric pressure across the tropical Pacific Ocean (McGregor and Ebi 2018). This phenomenon has direct implications for coffee production, as evidenced in the 2016 season crop when Espírito Santo, the largest Conilon-producing state in Brazil, recorded a reduction of 35.1% in coffee production (Conab 2016). This decline was attributed to the combination of drought conditions and high temperatures (annual average above 26 °C) (Venancio et al. 2020).

Abiotic stresses like that imposed by climate change significantly disrupt plant physiology at molecular, biochemical, and morphological levels, often causing irreversible damage (Borgo et al. 2025). Heat stress promotes the degradation of photosynthetic pigments and, when combined with water deficits, reduces transpiration, creating an ambient favorable for reactive oxygen species (ROS) accumulation (Lima et al. 2002; DaMatta and Ramalho 2006; DaMatta et al. 2018). ROS disrupts cellular redox homeostasis, causing oxidative damage to biomolecules (*e.g.*, lipids, proteins, DNA, and carbohydrates), which can reduce crop development and yield (Hasanuzzaman et al. 2020; Lanza and Reis 2021). However, some strategies can be employed to mitigate the damage induced by abiotic stress in Conilon production.

Adopting agroforestry systems, building water reservoirs, managing irrigation, and developing new cultivars are some strategies to mitigate the risks of coffee yield losses due to adverse climatic conditions (Pham et al. 2019; Venancio et al. 2020). Besides these strategies, plant nutrition is a powerful tool to mitigate the stress induced by abiotic factors in coffee plants (Borgo et al. 2025). Selenium (Se) is a beneficial element for plants, and when applied at low concentrations (Chauhan et al. 2019; Lanza and Reis 2021), it plays a crucial role in antioxidant metabolism, reducing oxidative stress caused by ROS (Gupta and Gupta 2017; Chauhan et al. 2019; Rengel et al. 2022). Additionally, proper Se application enhances photosynthetic activity by protecting the antenna complex, promoting greater starch and sugar pools that contribute to plant growth and development (Lanza and Reis 2021; Yang et al. 2022).

In Arabica coffee, foliar application of Se reduced oxidative stress, increased the activity of antioxidant enzymes [superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11), and catalase (CAT, EC 1.11.1.6)], enhanced the levels of photosynthetic pigments (chlorophylls, pheophytins, and carotenoids), and promoted yield increases between 38% and 42% compared with the control (Mateus et al. 2021). In Conilon coffee, foliar Se application reduced cold stress-induced leaf damage by 24% in seedlings grown under controlled conditions (Sousa et al. 2022). Some findings have pointed out that early foliar Se application, performed 8 days before stress incidence, significantly reduces oxidative stress in Arabica coffee seedlings (Sousa et al. 2023). However, to the best of our knowledge, no published studies have assessed the impact of foliar Se application—considering different sources and rates—on Conilon coffee production under field conditions. This represents a critical gap in our understanding that needs to be addressed.

Foliar Se application is an efficient approach for decreasing soil adsorption and root accumulation (Lanza and Reis 2021). However, the range between the optimal and toxic levels of Se is narrow and varies among species (Feng et al. 2013; White 2018). Therefore, research is needed to establish the ideal Se rate for each species under different edaphoclimatic conditions (Lanza and Reis 2021). Furthermore, applying different Se sources can cause toxicity depending on their chemical composition (Mateus et al. 2021). In general, most studies have focused on the use of inorganic Se sources with rapid release, such as sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4) (Silva et al. 2020b; Lanza and Reis 2021). However, a recent study has demonstrated the great potential of using the organic source Se-glycine, which is released more slowly and presents a lower risk of causing Se toxicity in plants (Li et al. 2023). From this context, our objectives with this study were to evaluate the effectiveness of different sources and rates of foliar-applied Se in mitigating stress and Conilon coffee production during the ENSO phenomenon by measuring the crop's nutritional, physiological, and productive parameters.

2 Materials and Methods

2.1 Experimental sites

The study was conducted in Linhares, Espírito Santo, Brazil, at two sites cultivated with Conilon coffee—site 1 (Latitude 19°18'01" S, Longitude 40°26'27" W) and site 2 (Latitude 19°18'18" S, Longitude 40°26'57" W)—to assess the consistency of Conilon coffee's response to foliar Se application. The experimental sites were established in 2018 with the Diamante Incaper 8112 cultivar, using 3.0 x 1.0 m spacing. During the growing cycle, irrigation and fertilization were managed using a drip irrigation system. Plants were managed using the Programmed Cycle Pruning (PCP), as described by Ferrão et al. (2019).

The soils at both study sites are classified as Latossolo Vermelho-Amarelo (LVA) with a clayey texture, according to the Brazilian Soil Classification System (SiBICS) (Santos et al. 2018). This classification corresponds to Xanthic Ferralsols in the World Reference Base for Soil Resources (WRB/FAO) (IUSS Working Group 2022). Soil samples were collected at depths of 0.0-0.2 m and 0.2-0.4 m. The physical and chemical properties were determined using methods described by Teixeira et al. (2017) (Table 1). The study was conducted during the 2023/2024 season, marked by the ENSO phenomenon. During this period, a total precipitation of 996.20 mm and an average temperature of 25.18 °C were recorded (INMET 2024) (Fig. 1).

2.2 Experimental design and treatments

The experiments were conducted simultaneously in a randomized block design, with a 2×5 factorial scheme with four replications. Two Se sources (sodium selenate: Se(VI) and Se-bisglycinate: Se-Bis) were applied via foliar sprays at five Se rates (0, 10, 20, 40, and 60 mg L^{-1}). The Se rates were determined based on the results obtained by Mateus et al. (2021). Each experimental plot consisted of lines of ten plants, but only the four central plants were evaluated. The treatments were applied when the fruits reached the small green fruit stage (Fig. 1). The foliar application was performed in the morning period using a motorized backpack sprayer under the following climatic conditions: wind speed between 3 and 10 km h^{-1}, relative humidity greater than 60%, and air temperature below 30 ºC.

2.3 Leaf sampling and evaluations

Two diagnostic leaf samples from the coffee plants (third leaf pair from the apex of the plagiotropic branches located in the middle third of the plant) were collected twenty days after the application of the treatments (Ferrão et al. 2019). In the first sampling, the collected leaves were immediately stored in liquid nitrogen (-196 ºC). The samples were ground in a porcelain mortar with liquid nitrogen and stored in an ultra-freezer (-80 ºC) until analysis. In the second sampling, the collected leaves were placed in pre-labeled paper bags and dried in a forced-air circulation oven (65 ± 5 °C) until they reached constant weight. After drying, the samples were ground in a Willey mill with a 40-mesh sieve.

The fresh tissue was used to assess the oxidative stress ENSO-induced by measuring hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) concentrations and the activities of enzymes that composes the antioxidant system (SOD, APX, and CAT). The dry tissues were used to determine the contents of Se, macronutrients [nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S)], micronutrients [iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), and boron (B)], carbohydrates [starch, sucrose, and total soluble sugars (TSS)], total soluble proteins (TSP), total free amino acids (AA), and proline, as described below.

2.3.1 Leaf content of Se, macronutrients, and micronutrients

The N contents were determined after sulfuric acid digestion by the Kjeldahl method using steam distillation (Malavolta et al. 1997). The contents of Se (mg kg^{-1}), macronutrients (N, P, K, Ca, Mg, and S - g kg^{-1}), and micronutrients (Fe, Cu, Zn, Mn, and B - mg kg^{-1}) in the leaves were determined following acid digestion methods, as described by Malavolta et al. (1997) and adapted by Silva Junior et al. (2017). The resulting extracts were analyzed using an inductively coupled plasma-optical emission spectrometer (ICP-OES). A blank sample and a certified reference material (White Clover – BCR 402, Institute for Reference Materials and Measurements – IRMM, Geel, Belgium) were included in each analysis batch to ensure result reliability.

2.3.2 Gas exchange

The physiological parameters of gas exchange were determined using a portable infrared gas analyzer (IRGA, Licor 6400XT) between 8:00 and 11:00 a.m. on clear days. The photosynthetically active radiation was standardized to 1000 μmol (photons) m^{-2} s^{-1}, and the

CO_2 concentration in the chamber was set to 400 ppm (Silva et al. 2022). Measurements were taken on fully expanded and healthy diagnostic leaves. The following parameters were evaluated: net CO_2 assimilation rate (*A*; µmol CO_2 m^{-2} s^{-1}), stomatal conductance (*gs*; mol H_2O m^{-2} s^{-1}), and transpiration rate (*E*; mmol H_2O m^{-2} s^{-1}).

2.3.3 Oxidative stress and antioxidant enzyme activity

The H_2O_2 concentration (µmol H_2O_2 g^{-1} FW) in the leaf tissue was determined following the methodology described by Gay et al. (1999). Lipid peroxidation analysis was performed by measuring MDA concentration (nmol g^{-1} FW), as proposed by Buege and Aust (1978). For the antioxidant system metabolism analysis, the frozen leaf tissue was weighed (0.3 g) and homogenized with a 50 mM potassium phosphate buffer solution (pH 7.5 + 1 mM EDTA + 1 mM phenylmethylsulfonyl fluoride (PMSF) + 5 mM ascorbate). The extracts were then centrifuged at 14,000 × g for 10 minutes at 4 °C. After centrifugation, the supernatant was collected and used to evaluate the activity of SOD (U SOD mg $protein^{-1}$), APX (nmol min^{-1} mg $protein^{-1}$), and CAT (nmol H_2O_2 min^{-1} mg $protein^{-1}$), as described by García-Limones et al. (2002) and Silva et al. (2020b).

2.3.4 Total soluble proteins, total free amino acids, proline, and carbohydrates

Leaf dry matter samples were weighed (0.2 g) and subjected to sequential extraction using ethanol at 100%, 80%, and 50%, according to López-Hidalgo et al. (2021). The TSP concentration (mg protein g^{-1} DW) was then determined following Bradford (1976). The AA (mmol aa g^{-1} DW) were quantified using the ninhydrin method (Yemm et al. 1995). Proline concentration (µmol proline g^{-1} DW) was determined following the methodology proposed by Bates et al. (1973). The starch extraction was performed on the samples resulting from protein extraction, using potassium acetate buffer and the enzyme amyloglucosidase (Hendriks et al. 2003; Zanandrea et al. 2009). The concentration of starch (µg glucose g^{-1} DW), sucrose (µmol glucose g^{-1} DW), and TSS (µmol glucose g^{-1} DW) was determined by the anthrone method (Dische 1962). The samples' absorbance readings were performed using a microplate reader model Epoch with the Gen5 Data Analysis software interface (Bio Tek Instruments, Inc., Vermont, USA).

2.4 Harvest and post-harvest evaluations

The harvest was carried out manually when more than 90% of the fruits matured (cherry coffee) (Ferrão et al. 2019). The fruits from each plant were harvested, weighed, and placed in bags. A five-liter sample was collected, weighed, and sent for drying on a suspended terrace (natural coffee), with constant rotation, until the coffee reached a water content of 12%. After drying, the samples were reweighed before and after coffee processing. The processed coffee (green beans) was used for the evaluations described below.

2.4.1 Screen size classification

The size of the green beans (screen size) was assessed according to the Official Brazilian Coffee Classification (COB), regulated by Normative Instruction No. 8, dated June 11, 2003 (Brasil 2003). A 100 g sample of coffee passed through a set of progressively smaller sieves. The beans retained on sieves ≥ 16 (diameter ≥ 6.35 mm) were collected and weighed on a precision electronic balance (0.0001 g), with the results expressed as a percentage.

2.4.2 Yielding rate and yield

The yielding rate is defined as the ratio between the weight of processed coffee and the weight of coffee in the dried pod, and it is determined according to Eq. 1.

$$\text{Yielding rate } (\%) = \frac{\text{weight of processed sample (kg)}}{\text{weight of coffee in the dried pod (kg)}} \text{ x } 100 \qquad (1)$$

Yield was estimated according to Eq. 2.

$$\text{Yield (bags ha}^{-1}) = \frac{\text{production (kg plant}^{-1}) \text{ x n° plants ha}^{-1}}{60} \qquad (2)$$

2.5 Statistical analysis

The data were submitted to analysis of variance (ANOVA) using the F-test ($p \leq 0.05$). The interactions between the factors were assessed, and when significant, the interaction was decomposed; otherwise, the main effects were analyzed. The means were compared using Tukey's test ($p \leq 0.05$). Principal Component Analysis (PCA) was employed to identify patterns and evaluate the correlations among the variables in the dataset. All statistical analyses and graph creation were performed using R software (R Core Team 2024).

3 Results

3.1 Leaf content of Se, macronutrients, and micronutrients

Plants treated with Se-Bis showed significantly higher foliar Se contents than those treated with Se(VI) (Fig. 2). In site 1, Se-Bis and Se(VI) applied at 60 mg L^{-1} of Se increased foliar Se contents by 931.58% (19.60 mg kg^{-1}) and 384.21% (9.20 mg kg^{-1}) compared with the control, respectively (Fig. 2A). In site 2, Se-Bis and Se(VI) at 60 mg L^{-1} of Se enhanced foliar Se contents by 700% (16.80 mg kg^{-1}) and 271.43% (7.80 mg kg^{-1}) compared with the control, respectively (Fig. 2B). However, applying Se sources and rates did not significantly affect the foliar contents of macronutrients and micronutrients in Conilon coffee (Supplementary Table S1). For sites 1 and 2, the average values obtained were, respectively: 30.83 and 29.71 g N kg^{-1}; 1.61 and 1.26 g P kg^{-1}; 17.11 and 17.23 g K kg^{-1}; 15.58 and 15.92 g Ca kg^{-1}; 2.09 and 2.34 g Mg kg^{-1}; 2.03 and 2.26 g S kg^{-1}; 96.95 and 71.63 mg Fe kg^{-1}; 4.49 and 7.21 mg Cu kg^{-1}; 6.88 and 6.86 mg Zn kg^{-1}; 20.10 and 19.00 mg Mn kg^{-1}; 50.95 and 56.59 mg B kg^{-1}.

3.2 Gas Exchange

Selenium supply significantly affected the gas exchange parameters in Conilon coffee in both evaluated sites (Fig. 3). In site 1, Se-Bis and Se(VI) applied at 20 mg L^{-1} of Se resulted in the highest values of *A*, *gs*, and *E* compared with the control (Figs. 3A, 3C, and 3E). In site 2, Se-Bis and Se(VI) applied at 10, 20, 40, and 60 mg L^{-1} of Se significantly increased *A*, *gs*, and *E* compared with the control (Figs. 3B, 3D, and 3F).

3.4 Oxidative stress and antioxidant enzymatic activity

Applying low Se rates reduced oxidative stress in Conilon coffee (Fig. 4). In site 1, Se(VI) was more effective than Se-Bis in reducing H_2O_2 and MDA concentrations. In contrast, plants treated with Se-Bis exhibited significantly lower MDA concentrations in site 2. Selenium applied at 20 mg L^{-1} significantly reduced the concentration of H_2O_2 in site 2 (Fig. 4B). Selenium applied at 10, 20, and 40 mg L^{-1} reduced the concentration of MDA in both sites (Figs. 4C and 4D). However, applying Se at 60 mg L^{-1} promoted a pro-oxidant effect in both sites.

Antioxidant enzymatic activity was significantly affected by foliar Se application in Conilon coffee (Fig. 5). In site 1, the application of Se-Bis and Se(VI) at a rate of 20 mg L^{-1} of Se resulted in the highest SOD activity (Fig. 5A). In contrast, highest APX activity was observed with the application of Se(VI) at rates of 10 and 20 mg L^{-1} of Se (Fig. 5C). In site 2, SOD and APX showed highest activities with the application of Se(VI) at 10 mg L^{-1} of Se (Figs. 5B and 5D). For CAT, in site 1, the highest activity was observed when it was applied 10 and 20 mg L^{-1} of Se, while in site 2, all Se rates promoted greater activity compared with the control (Figs. 5E and 5F).

3.5 Total soluble proteins, total free amino acids, proline, and carbohydrates

Carbohydrates responded similarly to the application of Se-Bis and Se(VI) in Conilon coffee (Fig. 6). On the other hand, starch concentrations increased with the application of growing rates of Se, with the highest response observed at the rate of 60 mg L^{-1} (Figs. 6A and 6B). In site 2, the 20 mg L^{-1} rate promoted the highest sucrose concentration in the leaves (Fig. 6D). TSS was not influenced by Se application (Figs. 6E and 6F).

Foliar Se application significantly affected the concentrations of TSP, AA, and proline (Fig. 7). Regarding the sources, significant differences were observed only in TSP, with the highest response for Se-Bis (Fig. 7A). Regarding the rates, TSP concentrations increased progressively with increasing Se rates, reaching the highest response at the rate of 60 mg L^{-1} (Figs. 7A and 7B). On the other hand, the highest concentrations of AA were observed at the lower rates of Se (0, 10, and 20 mg L^{-1}) in site 1 (Fig. 7C). Proline showed the highest response at the rate of 40 mg L^{-1} of Se in site 2 (Fig. 7F). However, Se application did not affect proline and AA concentrations in sites 1 and 2, respectively (Figs. 7D and 7E).

3.6 Screen size, yielding rate, and yield

The foliar application of Se positively affected the size of Conilon coffee beans, increasing in screen size (Figs. 8A and 8B). In general, the application of Se-Bis promoted a superior screen size compared to Se(VI) in both evaluated sites. For both Se sources, rates of 20 and 60 mg L^{-1} of Se resulted in the highest average screen sizes in sites 1 and 2, respectively. On the other hand, the application of Se did not significantly affect the yielding rate of Conilon coffee, with average values of 49.58% in site 1 and 59.46% in site 2 (Figs. 8C and 8D).

The foliar application of Se promoted a significant increase in the yield of Conilon coffee in both evaluated sites. In site 1, the Se sources resulted in similar responses for yield.

However, all applied rates increased yield compared with the control, with the rate of 20 mg L^{-1} showing the most significant response, with an average increase of 21.60% (11.17 bags) (Fig. 8E). In site 2, both the sources and rates of Se affected the yield of Conilon coffee. Se-Bis promoted an average increase of 12.75 bags compared to Se(VI). The 20 mg L^{-1} rate also promoted the greatest response, with an average increase of 13.77% in yield (20.08 bags) compared with the control (Fig. 8F).

3.6 Principal Component Analysis

In site 1, the foliar Se content showed a positive correlation with Se rates, starch, TSP, SOD, *A*, *E*, *gs*, yielding rate, and yield, and a negative correlation with AA, proline, H_2O_2, and APX levels. In site 2, a positive correlation was observed between foliar Se content and Se rates, starch, TSP, CAT, MDA, proline, *A*, *E*, *gs*, screen size, yielding rate, and yield (Fig. 9).

4 Discussion

Drought and unfavorable temperatures are the main climatic limitations of coffee production (DaMatta and Ramalho 2006; Borgo et al. 2025). The average temperatures exceeded the historical averages from 2012 to 2022 during the experiments. In November 2023, after foliar Se application, a sharp decrease in precipitation was observed, with a total of only 10.60 mm, accompanied by an increase of 1.58 °C in the average air temperature compared to the historical average (Fig. 1) (INMET 2024). In this study, we demonstrate that under these conditions, which were intensified by the ENSO phenomenon, the foliar application of Se was an effective strategy to mitigate oxidative stress (Fig. 4), maintain higher photosynthetic activity (Fig. 3), and ensure a greater yield of Conilon coffee (Fig. 8).

Foliar Se application increased the foliar Se contents in Conilon coffee (Fig. 2), corroborating the results obtained by Mateus et al. (2021). Despite the significant increase and the pro-oxidant effect observed with the application of 60 mg L^{-1} of Se (Fig. 4), the plants did not show visual symptoms of toxicity, regardless of the Se source applied. Furthermore, the increase in foliar Se contents did not result in significant changes in the macronutrient and micronutrient contents in the evaluated sites (Supplementary Table S1). Similarly, Mateus et al. (2020) did not observe visual symptoms of foliar toxicity in Arabica coffee genotypes (Obatã, IPR99, IAC125, IPR100, and Catucaí), which showed average increases of 100 times in Se contents in the shoots when grown in a nutrient solution containing Se (1.0 mmol L^{-1}). However, their findings pointed out that foliar contents of P and S decreased in plants exposed to high concentrations of Se, suggesting an ionic interaction effect (Fageria 2001; Zhou et al. 2020).

When subjected to environmental stresses, plants suffer damage to the chloroplasts, resulting in the disruption of photosynthesis (Feng et al. 2013). Heat and drought stress severely hampers stomatal conductance, plant water relations, CO_2 assimilation, and photosynthetic pigments, reducing crop yield (Chaudhry and Sidhu 2022). Our findings demonstrated that the proper application of Se can optimize the photosynthetic metabolism of Conilon coffee, promoting increases in stomatal conductance, transpiration, and CO_2 assimilation (Fig. 3). The PCA analysis confirmed that the applied rates of Se are directly related to the foliar contents of this element, which showed a positive correlation with the gas exchange parameters (*A*, *gs*, and *E*) in the two evaluated sites (Fig. 9). These findings reinforce the importance of Se application to enhance the photosynthetic performance of Conilon coffee under stress conditions.

The foliar application of Se-Bis and Se(VI) at 20 mg L^{-1} of Se showed the best results for *A*, *gs*, and *E* in Conilon coffee (Fig. 3). Applying Se at rates up to 40 mg L^{-1} in Arabica coffee increased the concentration of photosynthetic pigments, such as chlorophylls, pheophytins, and carotenoids. However, Se rates above 100 mg L^{-1}, in the form of sodium selenate, induced chlorophyll oxidation (Mateus et al. 2021). The combined effects of the biosynthesis of photoprotective pigments and the enhancement of the antioxidant metabolism induced by Se represent the main physiological mechanisms involved in the regulation of photosynthesis, contributing to increased photosynthetic performance in various plant species (Feng et al. 2013; Lanza and Reis 2021).

The overproduction of ROS leads to membrane lipid peroxidation, with MDA being one of the main markers used to quantify oxidative damage to lipids caused by environmental stresses (Hasanuzzaman et al. 2020; Lanza and Reis 2021). In our study, intermediate rates of Se (10, 20, and 40 mg L^{-1}) significantly reduced the foliar concentrations of MDA and H_2O_2 compared with the control (Fig. 4). These results can be attributed to the activation of antioxidative metabolism evidenced by the increase in the activities of SOD, APX, and CAT, which showed greatest responses with the application of 10 and 20 mg L^{-1} of Se (Fig. 5). These enzymes are responsible for eliminating ROS, protecting the plant cell membrane from oxidation (Silva et al. 2020b; Lanza and Reis 2021). SOD is a metalloenzyme that shows the frontline defense under excessive ROS generation by initiating the conversion of superoxide radical ($O_2^{\bullet -}$) into H_2O_2, which is further transformed into H_2O by CAT and APX (Hasanuzzaman et al. 2020). According to Mateus et al. (2021), the foliar application of Se at low rates increased the antioxidant enzyme activities and reduced ROS accumulation in coffee plants.

Regarding carbohydrates, the foliar concentrations were similar when applying Se-Bis and Se(VI). Applying 20 mg L^{-1} of Se resulted in a higher sucrose concentration than control, although this effect was inconsistent across the two evaluated sites. On the other hand, a direct relationship was observed between Se rates applied and the foliar starch concentration in both evaluated sites (Fig. 6). These results suggest that the reduction of oxidative stress, associated with the increased activity of antioxidant enzymes, contributed to greater photosynthetic performance, promoting starch accumulation in coffee leaves. This relationship is corroborated by the PCA analysis, which showed a positive correlation between the rates and the Se contents with the gas exchange parameters (*A*, *gs*, and *E*) and the foliar starch concentration (Fig. 9). Similar results were reported by Sousa et al. (2022), who identified a positive correlation between Se content and starch concentration in the leaves of Arabica (r = 0.92) and Conilon (r = 0.68) coffee. Furthermore, after subjecting both coffee species to cold stress, the authors observed a 30.7% increase in starch concentration in Se-treated plants.

The presence of starch in tissues and organs profoundly impacts the growing plant's physiology, as its synthesis and degradation govern the availability of free sugars, which control various growth and developmental processes (MacNeill et al. 2017). Furthermore, sugar metabolism and carbon skeletons are essential to synthesizing numerous compounds involved in the antioxidative protection of plants under stress (Couée et al. 2006). Soluble sugars (*e.g.*, sucrose, glucose, and fructose) are involved in the responses to several stresses, acting as nutrient and metabolite signaling molecules that activate specific or hormone crosstalk transduction pathways, resulting in important modifications of gene expression and proteomic patterns (Couée et al. 2006; Keunen et al. 2013). Higher carbohydrate concentrations (Fig. 6) are associated with greater CO_2 assimilation (Fig. 3) and, consequently, lower yield losses under stress conditions (Fig. 8) due to the ability of these carbohydrates to provide substrates and structural elements (Lara et al. 2024; Santos et al. 2024).

Overall, the foliar concentrations of TSP showed a similar pattern to starch but were inversely proportional to the AA concentrations in site 01 (Fig. 7). In this site, the PCA analysis indicated a strong negative correlation between the applied Se rates and the AA concentrations (Fig. 9). Sousa et al. (2022) also observed a negative correlation between Se and AA levels before and during cold stress in coffee plants. Although the AA concentration was not significantly altered by Se application in site 02, the results obtained in site 01 suggest that Se supply stimulated the conversion of AA into TSP under stress conditions. In plants, Se is assimilated through the S metabolic pathway, being converted into selenocysteine (SeCys) and selenomethionine (SeMet), which participate in the formation of selenoproteins by the non-specific replacement of cysteine and methionine, respectively (Rengel et al. 2022). Selenoproteins act as powerful antioxidants in plant metabolism through the glutathione peroxidase (GSH) pathway, combating ROS generated under stress conditions (Lanza and Reis 2021). Thus, the increase in TSP levels with the foliar application of Se may be related to the mitigation of oxidative stress observed in Conilon coffee.

The amino acid proline often accumulates during plants' exposure to adverse environmental conditions, which is crucial in preventing oxidative damage caused by ROS (Rejeb et al. 2014). In this study, the foliar application of Se at a rate of 40 mg L^{-1} resulted in a significant increase in foliar proline concentration in site 2 (Fig. 7F). Similar results were reported by Sousa et al. (2023), who observed that foliar Se supply increased proline concentration in Arabica coffee plants under osmotic stress. Proline acts as a non-enzymatic antioxidant, which plays a key role in osmotic adjustment and maintenance of membrane function and plant adaptation to various stress conditions (Lanza and Reis 2021; Renzetti et al. 2024). Besides acting as an excellent osmolyte, proline plays three significant roles during stress: as a metal chelator, an antioxidative defense molecule, and a signaling molecule (Hayat et al. 2012).

This set of nutritional and physiological responses to the foliar Se application described above directly impacted the yield of Conilon coffee (Fig. 8). The PCA analysis indicated that, in both evaluated sites, yield showed a strong correlation with the gas exchange parameters (A, gs, and E), which were optimized by the foliar application of Se. Furthermore, considering that the yielding rate was not significantly altered by Se supply, the increase in grain size (screen size) was a determining factor for the yield gain, reinforcing the positive correlation between yield and screen size (Fig. 9). Selenium applied at 20 mg L^{-1} resulted in the highest yield gains, averaging 21.60% (11.17 bags) in site 1 and 13.77% (20.08 bags) in site 2 compared with the control. These results are consistent with the findings of Mateus et al. (2021), who observed a 38% increase in Arabica coffee yield with the foliar application of 20 mg L^{-1} of Se, using sodium selenate as the source. These results demonstrate that Se has a beneficial effect on Conilon coffee grown under abiotic stress conditions, acting to reduce oxidative stress and optimize photosynthetic performance, resulting in yield gain for the crop.

To the best of our knowledge, no published studies have investigated the effects of the foliar application of Se-Bis on stress mitigation and production in Conilon coffee under field conditions. Although most evaluated variables presented similar responses to both Se-Bis and Se(VI) applications in this study, Se-Bis demonstrated clear advantages in several key parameters. Specifically, it promoted significantly higher foliar Se contents (Fig. 2) and increased grain size at both evaluated sites (Figs. 8A and 8B), reinforcing the performance of Se-Bis as a Se source. Furthermore, the Se-Bis application resulted in a higher yield in site 2 (Fig. 8F). These findings highlight the considerable potential of Se-Bis as an organic source of Se for mitigating abiotic stresses in Conilon coffee. Overall, both Se-Bis and Se(VI) effectively supplied Se through foliar application and mitigated stress during the ENSO phenomenon, ensuring a higher yield than the Se application's absence.

According to the Intergovernmental Panel on Climate Change (IPCC) report, it is estimated that global temperatures may exceed 4 °C by 2100 compared to pre-industrial levels (IPCC 2021). As a perennial and climate-sensitive crop, coffee will be highly affected by climate change (DaMatta et al. 2018; Pham et al. 2019), with projections indicating a 25% reduction in yield by the end of the 21st century (Tavares et al. 2018). Considering that ENSO intensifies adverse climatic conditions in the central coffee-producing regions (Silva et al. 2020a), this study supports the foliar application of Se as an effective and promising approach to mitigating abiotic stresses and increasing yield gains in Conilon coffee.

5 Conclusion

The foliar application of Se-Bis and Se(VI) proved effective in supplying Se, significantly increasing foliar Se contents without causing visual toxicity symptoms or altering the contents of macronutrients (N, P, K, Ca, Mg, and S) and micronutrients (Fe, Cu, Zn, Mn, and B) in Conilon coffee plants. Selenium supply increased foliar proline concentration and antioxidant enzyme activity (SOD, APX, and CAT), reduced lipid peroxidation (MDA), and improved gas exchange parameters (*A*, *gs*, and *E*), promoting the accumulation of starch in the leaves. The 20 mg L^{-1} of Se rate resulted in the highest yield gains, averaging 21.60% (11.17 bags) and 13.77% (20.08 bags) compared with the control. Our findings suggest that the foliar application of Se stimulates the conversion of AA into TSP in Conilon coffee under abiotic stress conditions. Foliar application of Se(VI) and Se-Bis at 20 mg L^{-1} of Se during the small green fruit stage proved to be a practical approach for reducing oxidative stress, enhancing photosynthetic performance, and ensuring higher yield of Conilon coffee during ENSO and in the context of climate change.

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Declaration of Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Authors' contributions

All authors contributed intellectual input and assistance to this study and manuscript preparation.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Table 1 Physical and chemical characterization of soils at the study sites in Linhares, Espírito Santo, Brazil.

Sites	Depth (m)	Physical and chemical characterization
Site 1	0.0-0.2	OM = 3.53 dag kg^{-1}; pH = 6.30; Ca = 5.73 cmol$_c$ dm^{-3}; Mg = 1.94 cmol$_c$ dm^{-3}; K = 130.78 mg dm^{-3}; Al^{3+} = 0.20 cmol$_c$ dm^{-3}; H+Al = 3.10 cmol$_c$ dm^{-3}; SB = 8.01 cmol$_c$ dm^{-3}; CEC = 11.11 cmol$_c$ dm^{-3}; BS = 72.06%; P = 198.03 mg dm^{-3}; S-SO$_4^{2-}$ = 11.70 mg dm^{-3}; Zn = 12.10 mg dm^{-3}; Fe = 72.60 mg dm^{-3}; Mn = 14.80 mg dm^{-3}; Cu = 1.64 mg dm^{-3}; B = 0.40 mg dm^{-3}; sand = 400 g kg^{-1}; silt = 70 g kg^{-1}; clay = 530 g kg^{-1}.
	0.2-0.4	OM = 2.53 dag kg^{-1}; pH = 4.50; Ca = 1.79 cmol$_c$ dm^{-3}; Mg = 0.49 cmol$_c$ dm^{-3}; K = 61.99 mg dm^{-3}; Al^{3+} = 0.40 cmol$_c$ dm^{-3}; H+Al = 6.10 cmol$_c$ dm^{-3}; SB = 2.44 cmol$_c$ dm^{-3}; CEC = 8.54 cmol$_c$ dm^{-3}; BS = 28.56%; P = 24.71 mg dm^{-3}; S-SO$_4^{2-}$ = 95.70 mg dm^{-3}; Zn = 2.20 mg dm^{-3}; Fe = 141.40 mg dm^{-3}; Mn = 4.90 mg dm^{-3}; Cu = 0.47 mg dm^{-3}; B = 0.22 mg dm^{-3}; sand = 330 g kg^{-1}; silt = 120 g kg^{-1}; clay = 550 g kg^{-1}.
Site 2	0.0-0.2	OM = 4.13 dag kg^{-1}; pH = 6.60; Ca = 6.75 cmol$_c$ dm^{-3}; Mg = 1.44 cmol$_c$ dm^{-3}; K = 64.57 mg dm^{-3}; Al^{3+} = 0.20 cmol$_c$ dm^{-3}; H+Al = 2.00 cmol$_c$ dm^{-3}; SB = 8.36 cmol$_c$ dm^{-3}; CEC = 10.36 cmol$_c$ dm^{-3}; BS = 80.65%; P = 13.47 mg dm^{-3}; S-SO$_4^{2-}$ = 25.90 mg dm^{-3}; Zn = 14.00 mg dm^{-3}; Fe = 102.60 mg dm^{-3}; Mn = 10.60 mg dm^{-3}; Cu = 13.99 mg dm^{-3}; B = 0.22 mg dm^{-3}; sand = 410 g kg^{-1}; silt = 40 g kg^{-1}; clay = 550 g kg^{-1}.
	0.2-0.4	OM = 2.17 dag kg^{-1}; pH = 4.40; Ca = 0.87 cmol$_c$ dm^{-3}; Mg = 0.21 cmol$_c$ dm^{-3}; K = 72.73 mg dm^{-3}; Al^{3+} = 0.90 cmol$_c$ dm^{-3}; H+Al = 6.60 cmol$_c$ dm^{-3}; SB = 1.27 cmol$_c$ dm^{-3}; CEC = 7.87 cmol$_c$ dm^{-3}; BS = 16.09%; P = 12.96 mg dm^{-3}; S-SO$_4^{2-}$ = 107.80 mg dm^{-3}; Zn = 0.80 mg dm^{-3}; Fe = 166.70 mg dm^{-3}; Mn = 2.30 mg dm^{-3}; Cu = 0.92 mg dm^{-3}; B = 0.21 mg dm^{-3}; sand = 370 g kg^{-1}; silt = 100 g kg^{-1}; clay = 530 g kg^{-1}.

pH (H_2O); organic matter (OM) (wet oxidation with $Na_2Cr_2O_7$ 4 N + H_2SO_4 10 N); sum of bases (SB); cation exchange capacity at pH 7 (CEC); base saturation (BS); P, K, Fe, Zn, Mn, and Cu (extractant Mehlich-1); Ca, Mg, and Al^{3+} (extractant KCl 1 mol L^{-1}); H+Al (extractant SMP); B (extractant hot water); S-SO$_4^{2-}$ (extractant monocalcium phosphate).

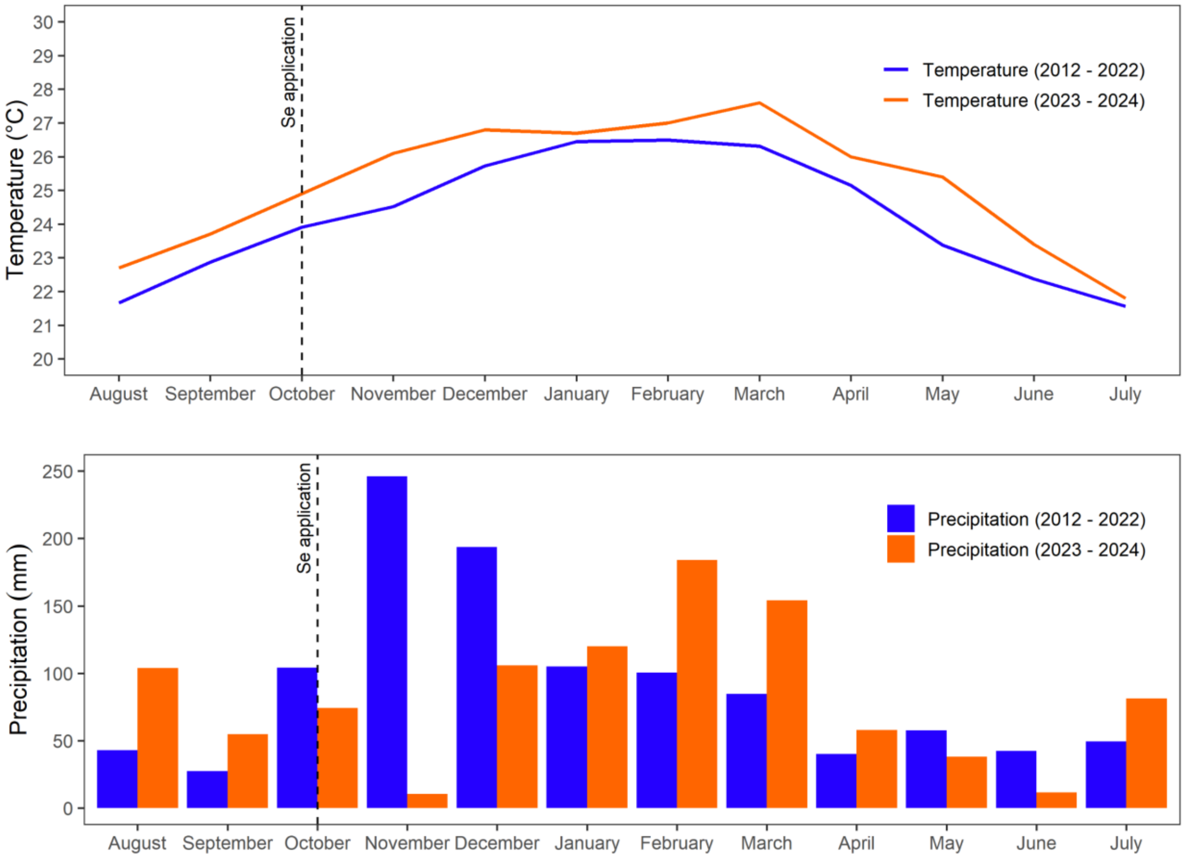

Fig. 1 Monthly averages of temperature and accumulated precipitation (2012 to 2022 and 2023 to 2024) recorded in Linhares, Espírito Santo, Brazil. The dashed lines indicate the month of foliar Se application on Conilon coffee (*C. canephora* cv. Diamante Incaper 8112).

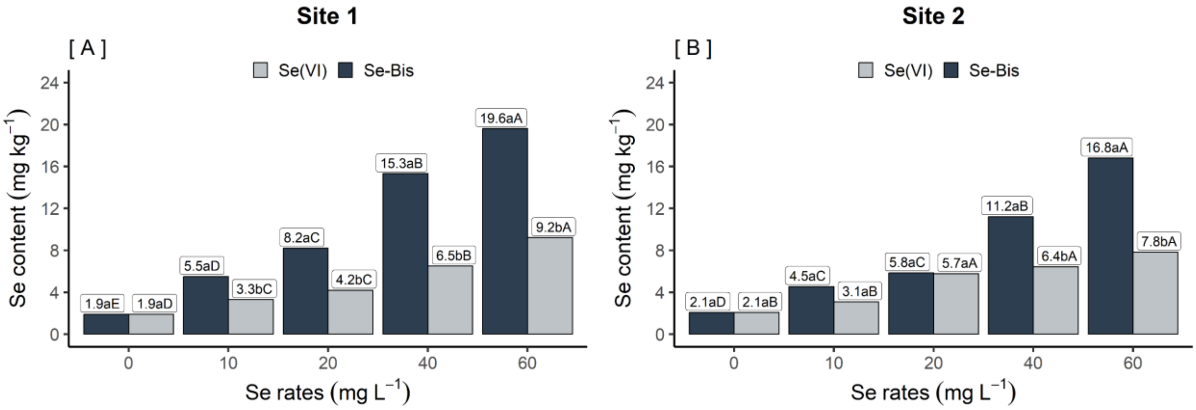

Fig. 2 Foliar Se content (A and B) in response to foliar application of Se(VI) and Se-Bis at different Se rates in Conilon coffee (*C. canephora* cv. Diamante Incaper 8112) cultivated at sites 1 and 2 in Linhares, Espírito Santo, Brazil. Means followed by the same lowercase letter (comparing sources) or uppercase letter (comparing rates) are not significantly different according to Tukey's test ($p \leq 0.05$).

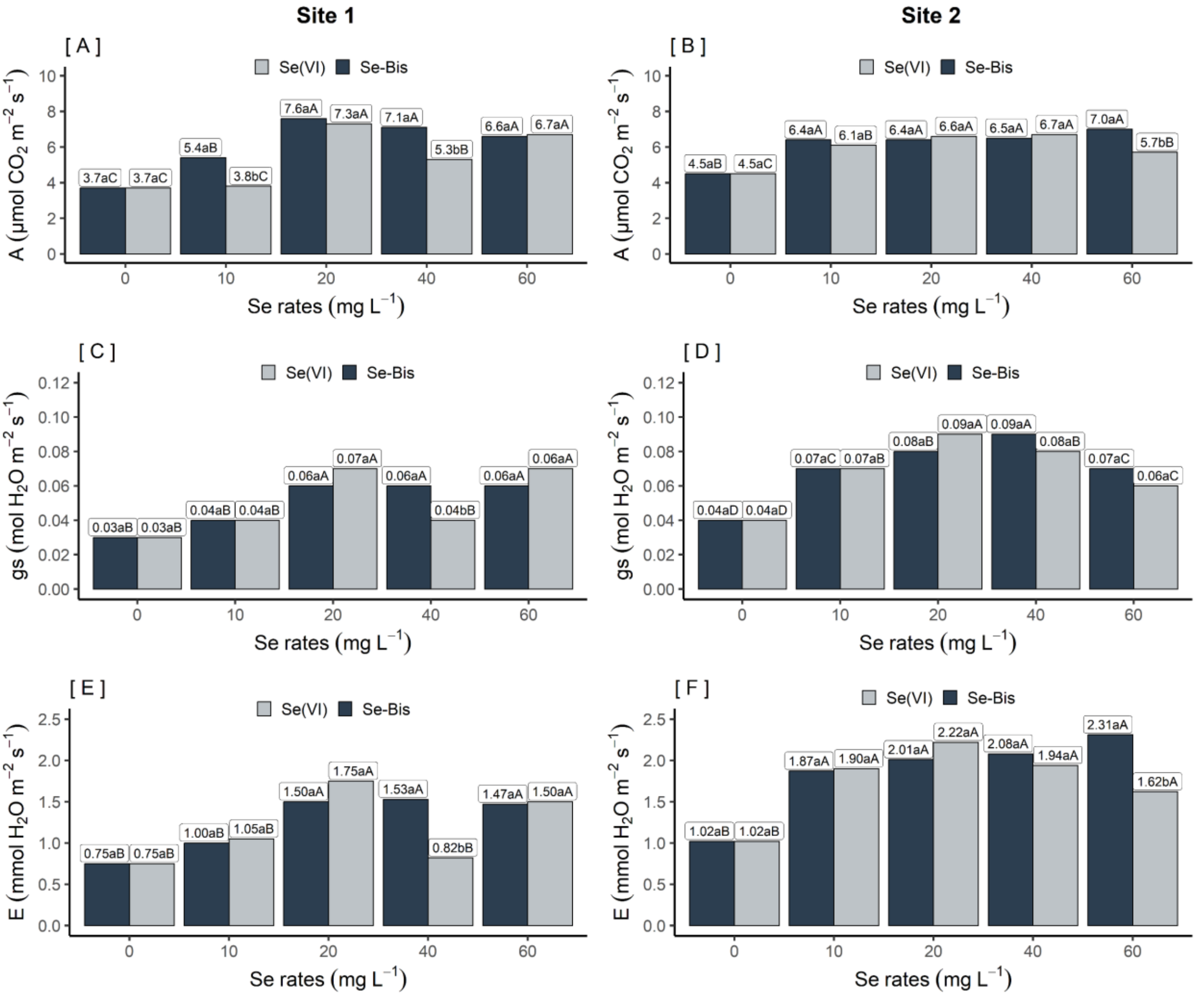

Fig. 3 Net CO_2 assimilation rate (*A*; A and B), stomatal conductance (*gs*; C and D), and transpiration rate (*E*; E and F) in response to foliar application of Se(VI) and Se-Bis at different Se rates in Conilon coffee (*C. canephora* cv. Diamante Incaper 8112) cultivated at sites 1 and 2 in Linhares, Espírito Santo, Brazil. Means followed by the same lowercase letter (comparing sources) or uppercase letter (comparing rates) are not significantly different according to Tukey's test ($p \leq 0.05$).

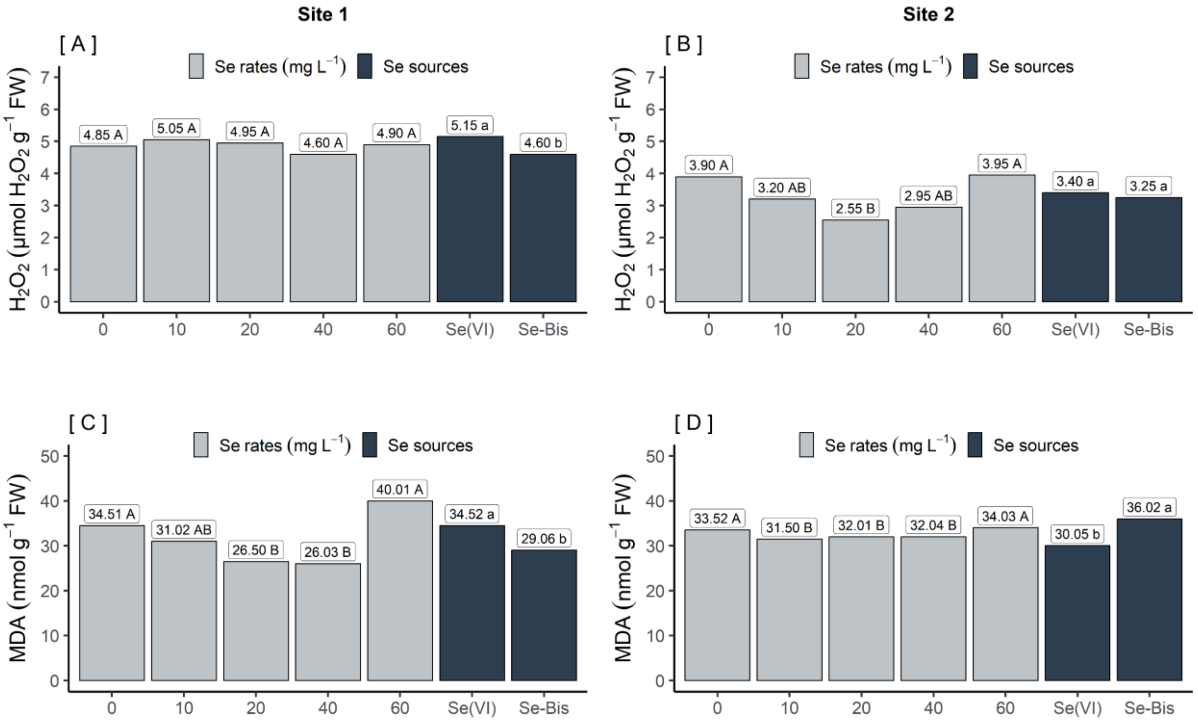

Fig. 4 Hydrogen peroxide (H_2O_2; A and B) and malondialdehyde (MDA; C and D) concentrations in response to foliar application of Se(VI) and Se-Bis at different Se rates in Conilon coffee (*C. canephora* cv. Diamante Incaper 8112) cultivated at sites 1 and 2 in Linhares, Espírito Santo, Brazil. Means followed by the same lowercase letter (comparing sources) or uppercase letter (comparing rates) are not significantly different according to Tukey's test ($p \leq 0.05$).

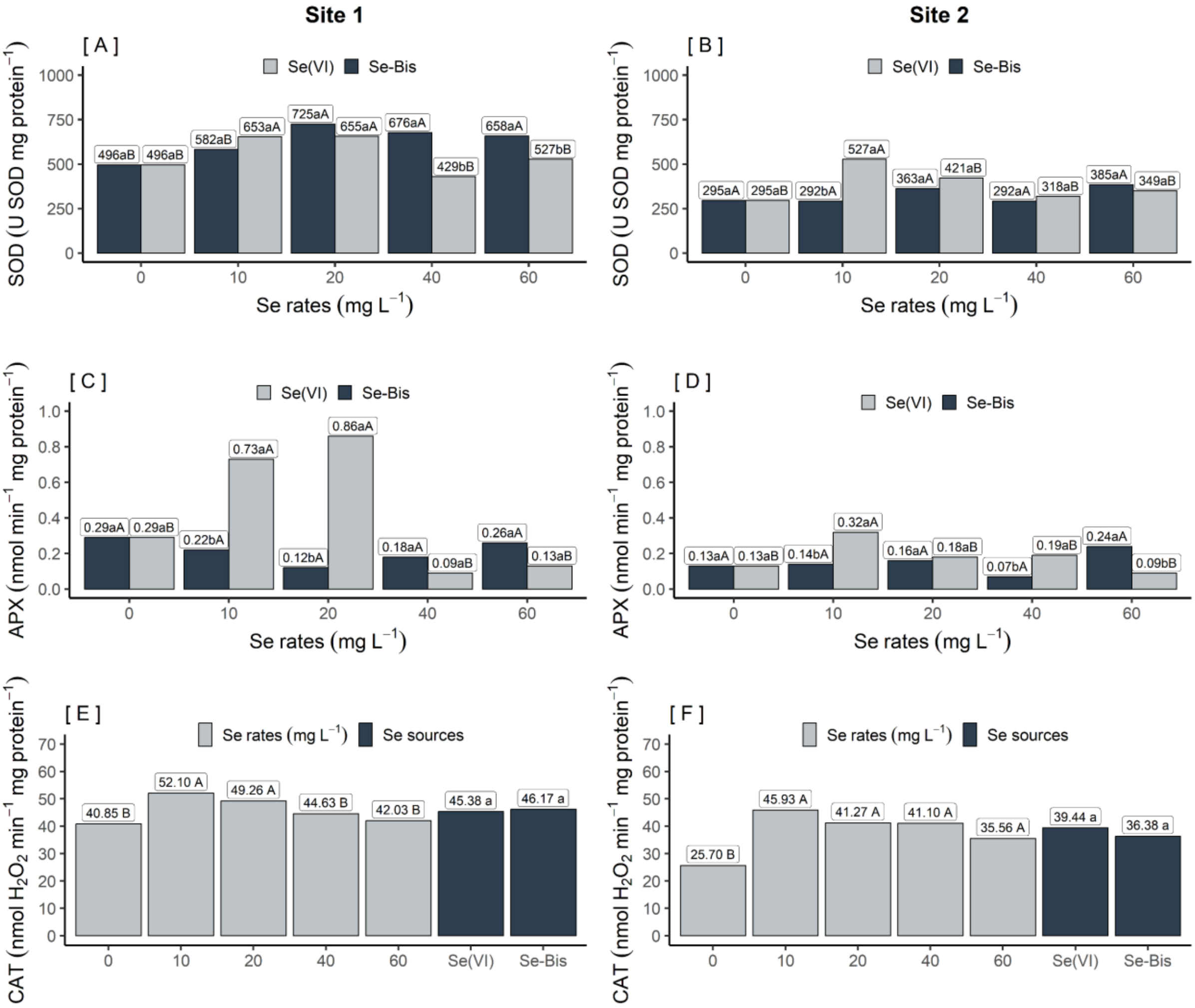

Fig. 5 Enzymatic activity of superoxide dismutase (SOD; A and B), ascorbate peroxidase (APX; C and D), and catalase (CAT; E and F) in response to foliar application of Se(VI) and Se-Bis at different Se rates in Conilon coffee (*C. canephora* cv. Diamante Incaper 8112) cultivated at sites 1 and 2 in Linhares, Espírito Santo, Brazil. Means followed by the same lowercase letter (comparing sources) or uppercase letter (comparing rates) are not significantly different according to Tukey's test ($p \leq 0.05$).

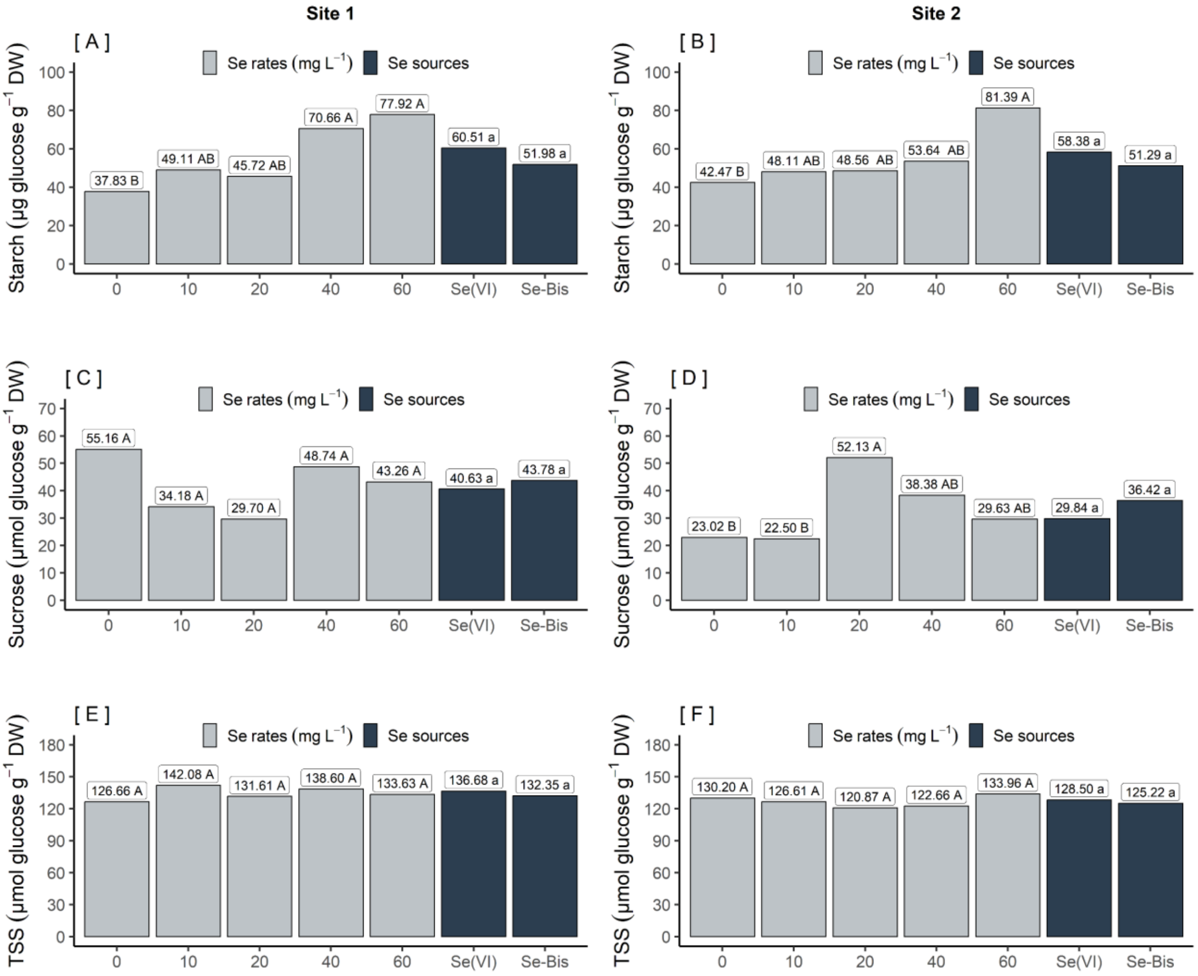

Fig. 6 Starch (A and B), sucrose (C and D), and total soluble sugars (TSS; E and F) concentrations in response to foliar application of Se(VI) and Se-Bis at different Se rates in Conilon coffee (*C. canephora* cv. Diamante Incaper 8112) cultivated at sites 1 and 2 in Linhares, Espírito Santo, Brazil. Means followed by the same lowercase letter (comparing sources) or uppercase letter (comparing rates) are not significantly different according to Tukey's test ($p \leq 0.05$).

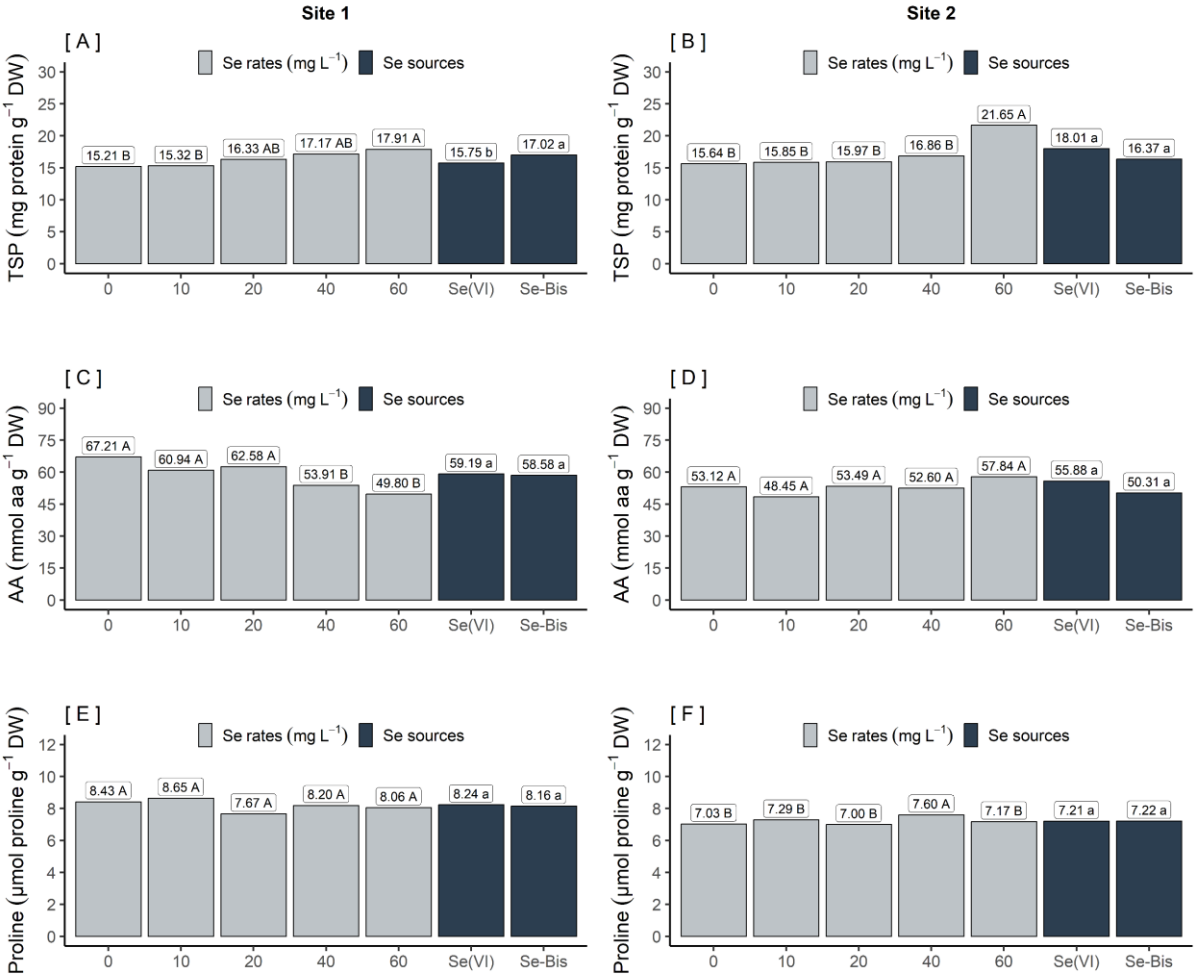

Fig. 7 Total soluble proteins (TSP; A and B), total free amino acids (AA; C and D), and proline (E and F) concentrations in response to foliar application of Se(VI) and Se-Bis at different Se rates in Conilon coffee (*C. canephora* cv. Diamante Incaper 8112) cultivated at sites 1 and 2 in Linhares, Espírito Santo, Brazil. Means followed by the same lowercase letter (comparing sources) or uppercase letter (comparing rates) are not significantly different according to Tukey's test ($p \leq 0.05$).

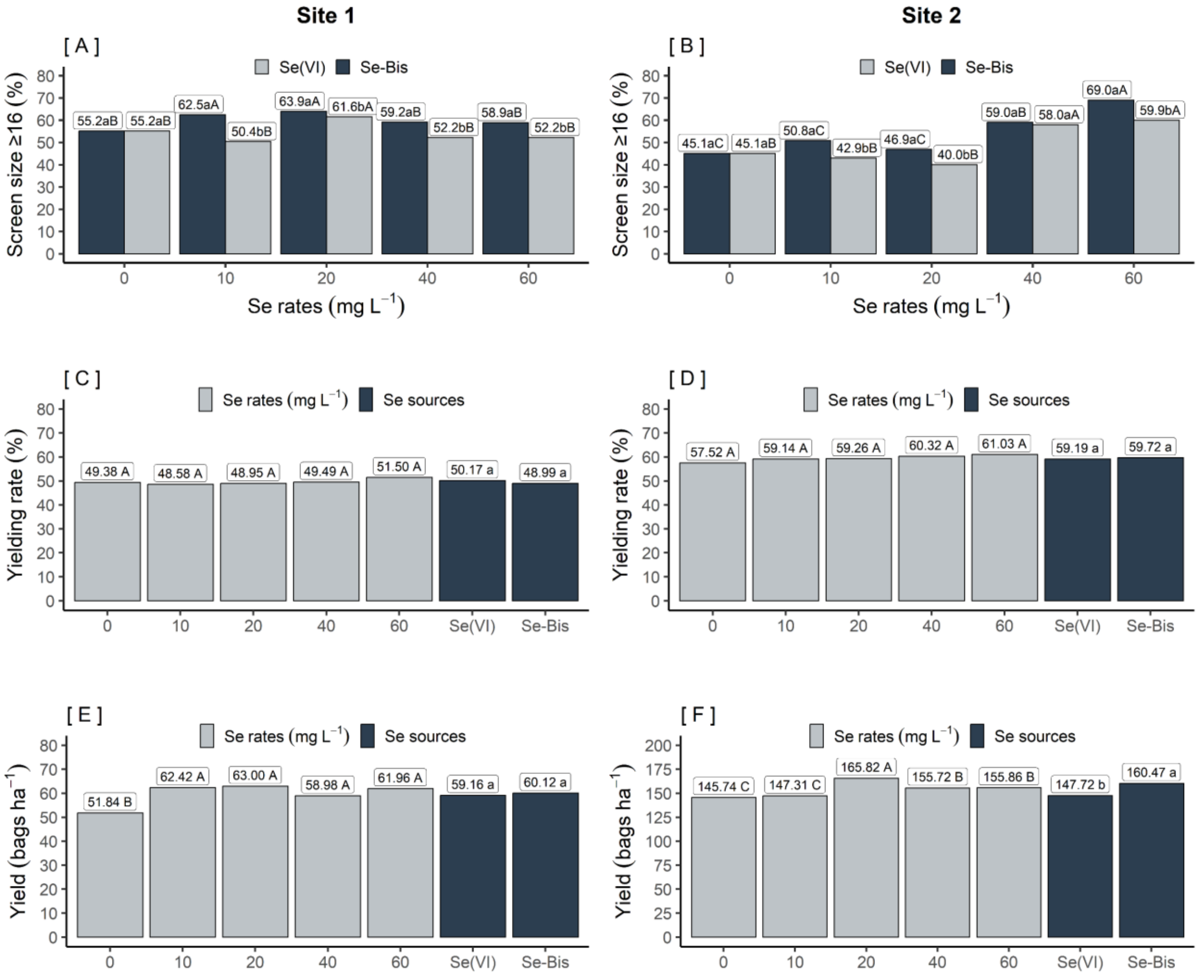

Fig. 8 Screen size ≥ 16 (A and B), yielding rate (C and D), and yield (E and F) of coffee beans in response to foliar application of Se(VI) and Se-Bis at different Se rates in Conilon coffee (*C. canephora* cv. Diamante Incaper 8112) cultivated at sites 1 and 2 in Linhares, Espírito Santo, Brazil. Means followed by the same lowercase letter (comparing sources) or uppercase letter (comparing rates) are not significantly different according to Tukey's test ($p \leq 0.05$).

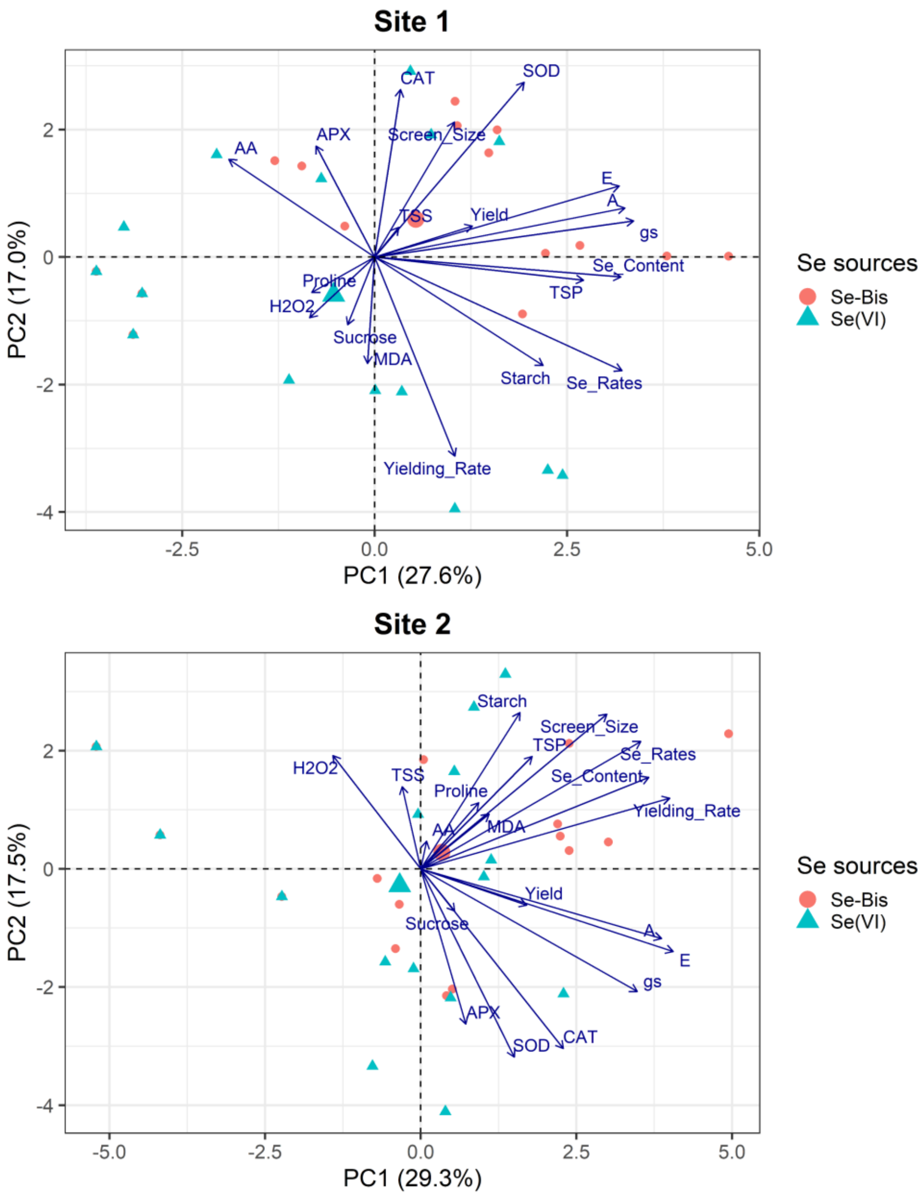

Fig. 9 Principal component analysis of Se rates (Se_Rates), foliar Se content (Se_Content), net CO_2 assimilation rate (A), stomatal conductance (gs), transpiration rate (E), hydrogen peroxide (H2O2), malondialdehyde (MDA), superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), starch (Starch), sucrose (Sucrose), total soluble sugars (TSS), total soluble proteins (TSP), total free amino acids (AA), proline (Proline), screen size ≥ 16 (Screen_Size), yielding rate (Yielding_Rate), and yield (Yield) in response to foliar application of Se(VI) and Se-Bis at different Se rates in Conilon coffee (*C. canephora* cv. Diamante Incaper 8112) cultivated at sites 1 and 2 in Linhares, Espírito Santo, Brazil.

Supplementary Table S1 Mean and standard deviation of foliar macronutrient (N, P, K, Ca, Mg, and S - g kg^{-1}) and micronutrient (Fe, Cu, Zn, Mn, and B - mg kg^{-1}) contents in response to foliar application of Se(VI) and Se-Bis at different Se rates in Conilon coffee (*C. canephora* cv. Diamante Incaper 8112) cultivated at sites 1 and 2 in Linhares, Espírito Santo, Brazil.

Site 1											
Sources	N^{ns}	P^{ns}	K^{ns}	Ca^{ns}	Mg^{ns}	S^{ns}	Fe^{ns}	Cu^{ns}	Zn^{ns}	Mn^{ns}	B^{ns}
Se-Bis	31.02 ± 1.4	1.61 ± 0.1	17.13 ± 1.8	15.66 ± 3.8	2.10 ± 0.3	2.06 ± 0.1	99.2 ± 18.0	5.31 ± 7.28	6.96 ± 0.60	21.03 ± 5.72	53.71 ± 18.25
Se(VI)	30.64 ± 1.3	1.61 ± 0.1	17.08 ± 1.8	15.51 ± 3.6	2.08 ± 0.4	2.00 ± 0.1	94.7 ± 16.9	3.66 ± 1.09	6.81 ± 0.62	19.17 ± 4.97	48.19 ± 8.32
Rates (mg L^{-1})	N^{ns}	P^{ns}	K^{ns}	Ca^{ns}	Mg^{ns}	S^{ns}	Fe^{ns}	Cu^{ns}	Zn^{ns}	Mn^{ns}	B^{ns}
0	32.03 ± 1.8	1.59 ± 0.1	16.32 ± 2.3	18.29 ± 6.9	2.11 ± 0.5	1.98 ± 0.1	86.82 ± 20.6	2.90 ± 0.65	6.92 ± 1.11	17.67 ± 4.45	50.34 ± 12.14
10	30.88 ± 0.9	1.63 ± 0.1	16.90 ± 2.2	15.49 ± 2.6	1.97 ± 0.4	2.02 ± 0.2	103.12 ± 23.9	8.44 ± 11.32	7.08 ± 0.45	18.80 ± 7.63	57.30 ± 9.25
20	30.65 ± 1.1	1.61 ± 0.1	16.30 ± 1.5	16.33 ± 1.7	2.28 ± 0.2	2.12 ± 0.1	109.02 ± 11.8	4.37 ± 1.36	7.12 ± 0.29	20.19 ± 4.50	51.52 ± 8.05
40	29.95 ± 1.0	1.61 ± 0.1	17.71 ± 1.4	13.62 ± 1.8	1.98 ± 0.3	2.00 ± 0.1	88.90 ± 9.9	3.46 ± 0.39	6.52 ± 0.30	20.02 ± 4.59	47.07 ± 3.59
60	30.63 ± 1.4	1.61 ± 0.1	18.31 ± 1.2	14.19 ± 1.3	2.10 ± 0.3	2.03 ± 0.1	96.90 ± 8.7	3.25 ± 0.60	6.77 ± 0.46	23.82 ± 4.60	48.53 ± 4.10
Site 2											
Sources	N^{ns}	P^{ns}	K^{ns}	Ca^{ns}	Mg^{ns}	S^{ns}	Fe^{ns}	Cu^{ns}	Zn^{ns}	Mn^{ns}	B^{ns}
Se-Bis	29.82 ± 1.0	1.29 ± 0.1	17.52 ± 1.1	15.89 ± 2.9	2.35 ± 0.3	2.26 ± 0.2	71.58 ± 7.3	7.18 ± 3.2	6.86 ± 1.3	18.31 ± 4.5	51.45 ± 4.7
Se(VI)	29.60 ± 1.2	1.23 ± 0.1	16.93 ± 2.3	15.96 ± 2.4	2.33 ± 0.4	2.26 ± 0.2	71.69 ± 11.2	7.24 ± 3.2	6.87 ± 1.2	19.69 ± 3.9	61.73 ± 14.2
Rates (mg L^{-1})	N^{ns}	P^{ns}	K^{ns}	Ca^{ns}	Mg^{ns}	S^{ns}	Fe^{ns}	Cu^{ns}	Zn^{ns}	Mn^{ns}	B^{ns}
0	29.73 ± 1.5	1.19 ± 0.1	17.44 ± 1.0	13.94 ± 0.4	2.30 ± 0.1	2.22 ± 0.1	72.94 ± 6.5	9.41 ± 6.2	6.11 ± 0.2	21.08 ± 2.3	67.84 ± 22.5
10	30.42 ± 0.9	1.29 ± 0.1	18.14 ± 1.4	17.20 ± 3.3	2.48 ± 0.3	2.38 ± 0.2	77.30 ±12.3	5.30 ± 0.7	7.26 ± 1.8	19.34 ± 5.0	56.01 ± 6.0
20	29.20 ± 1.1	1.26 ± 0.1	16.11 ± 2.6	16.05 ± 2.9	2.18 ± 0.4	2.15 ± 0.2	65.21 ± 8.9	7.03 ± 2.3	7.19 ± 1.4	18.12 ± 5.9	48.97 ± 8.6
40	29.08 ± 0.9	1.26 ± 0.1	17.50 ± 1.3	15.60 ± 1.1	2.34 ± 0.4	2.27 ± 0.1	73.44 ± 8.4	6.80 ± 1.7	6.42 ± 0.2	17.47 ± 4.9	53.44 ± 9.2
60	30.12 ± 0.7	1.32 ± 0.2	16.96 ± 2.1	16.83 ± 3.4	2.40 ± 0.3	2.29 ± 0.2	69.28 ± 7.6	7.50 ± 0.8	7.33 ± 1.5	19.00 ± 2.2	56.67 ± 8.5

Ns: No significant response by the F test ($p \leq 0.05$).

Article 2 – Written following the scientific journal's guidelines – Submitted version	
Article title:	Agronomic Biofortification with Selenium Enhances Conilon Coffee Quality and Contributes to Human Selenium Intake: A Study of Selenium Sources and Rates
Authors:	Edinei José Armani Borghi Fabrício Teixeira de Lima Gomes Leônidas Canuto dos Santos Amanda Santana Chales Mateus Portes Dutra Euler dos Santos Silva Maria Ligia de Souza Silva André Rodrigues dos Reis Flávio Henrique Silveira Rabêlo Tassio da Silva de Souza
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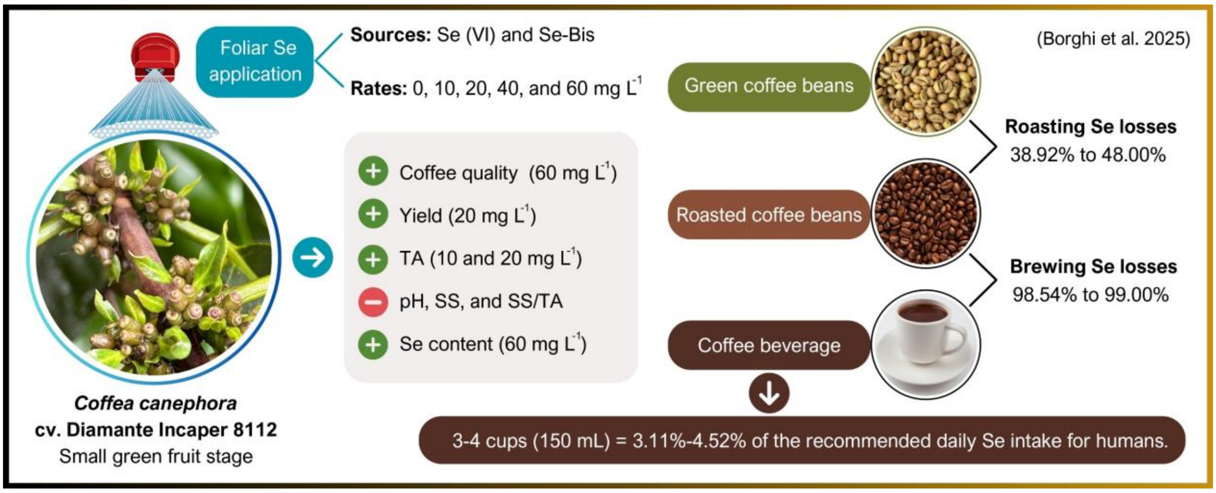
Article 2 – Agronomic Biofortification with Selenium Enhances Conilon Coffee Quality and Contributes to Human Selenium Intake: A Study of Selenium Sources and Rates

Abstract

Selenium (Se) deficiency in human beings is a global concern. Biofortification is an effective strategy to enhance the nutritional value of food and mitigate Se-hidden hunger. This study evaluated the biofortification of Conilon coffee beans by testing the foliar application of different Se sources and rates and assessing their effects on the physicochemical attributes of the beans, yield, and beverage quality. Two experiments were conducted using a randomized block design with a 2×5 factorial arrangement and four replications. Sodium selenate (Na_2SeO_4 – Se(VI)) and Se-bisglycinate (Se-Bis) were applied at five Se rates (0, 10, 20, 40, and 60 mg L^{-1}) during the small green fruit stage. Foliar Se application at 60 mg L^{-1} enhanced coffee quality and increased Se concentration in green and roasted beans, contributing to 3.11%–4.52% (3–4 cups day^{-1}) of the recommended daily intake for adults. Selenium application up to 60 mg L^{-1}, using Se(VI) and Se-Bis, effectively biofortified coffee without inducing visual toxicity symptoms. On the other hand, significant Se losses occurred during roasting (38.92%–48.00%) and brewing (98.54%–99.00%). Our findings indicate that Se biofortification enhances the quality of Conilon coffee without compromising yield, offering a promising strategy for increasing Se intake in the diet. Further studies are needed to explore how Se application impacts the concentration of physicochemical compounds in beans and beverage quality in Conilon coffee.

Keywords *Coffea canephora*. Hidden hunger. Food security. Sensory analysis.



1 Introduction

Approximately one-third of the world’s population, particularly in developing countries, suffers from micronutrient malnutrition—commonly referred to as “hidden hunger”—which includes deficiencies in selenium (Se), iron (Fe), zinc (Zn), iodine (I), lysine, folic acid, and vitamins A, C, D, and B12 (WHO, 2024; 2025). Selenium is an essential element for both animals and humans, required for synthesizing more than 25 selenoproteins, which have a wide range of pleiotropic effects, including antioxidant and anti-inflammatory properties, as well as the production of active thyroid hormones (Rayman, 2012). However, Se deficiency in the human diet remains a global concern, with significant health implications (Schiavon et al., 2020). Low Se intake in the diet is associated with various health problems, including higher mortality, increased cancer risk, impaired immune function, increased viral virulence, Keshan disease, Kashin-Beck disease, autoimmune thyroid disease, fertility/reproduction issues, cognitive decline, and type 2 diabetes (Fairweather-Tait et al., 2011; Rayman, 2020).

Considering the importance of adequate Se intake in the diet and the significant deficiency of this element in the global population, it is necessary to adopt strategies to increase its content in food to combat malnutrition (Boldrin et al., 2013). Increasing the nutritional value of staple crops through biofortification is a feasible and effective strategy to combat hidden hunger (Bouis and Saltzman, 2017). Agronomic biofortification aims to enhance the nutritional value of food through the optimized application of fertilizers (Cakmak, 2008; Cakmak and Kutman, 2018). Several studies have shown that Se biofortification can enhance crop yield, improve the nutritional and biochemical quality of food, and contribute to greater Se intake in the human diet (Lanza and Reis, 2021; Schiavon et al., 2020). Agronomic biofortification studies should focus on foods or beverages widely consumed by the population (Boldrin et al., 2013; Mateus et al., 2021). Coffee is one of the most consumed beverages globally, with increasing demand in nearly every region (FAO, 2025). Furthermore, coffee consumption plays a key role in human health by reducing the mortality rate associated with various causes (*e.g.*, diabetes mellitus, dementia, Parkinson's disease, cardiovascular diseases, and cancers) (Barrea et al., 2021; Safe et al., 2023). Therefore, agronomic biofortification of coffee is a promising approach to increasing Se dietary intake and enhancing the beverage's health benefits.

Brazil is the world's largest producer and exporter of coffee, ranking second among the largest beverage consumers (USDA, 2024). The production of foods with adequate Se concentration is crucial in Brazil, given the country's significant role as a global food supplier (Araujo et al., 2020). However, most agricultural soils in Brazil have low Se concentration, limiting crop uptake and reducing its presence in the human diet (Carvalho et al., 2019; Lopes et al., 2017; Reis et al., 2017). The availability of Se in soils depends on several factors, including the chemical form of the element, organic matter, soil mineralogy, redox condition, pH, and the presence of other anions (Lopes et al., 2017; Dinh et al., 2019). Tropical soils, such as those in Brazil, contain significant amounts of 1:1 clays (kaolinite) and Fe and Al oxides, which have a high capacity to adsorb anions such as phosphate, selenite, and selenate, thereby reducing Se availability in the soil solution (Araujo et al., 2018). In this case, foliar Se application is an effective strategy to address this issue and supply Se to plants (Lanza and Reis, 2021).

Foliar Se application has proven to be an effective strategy for increasing Se concentration in Arabica coffee beans (*Coffea arabica* L.) (Mateus et al., 2021). However, to the best of our knowledge, there are no published studies that have evaluated the impact of foliar Se application—considering different sources and rates—on the biofortification of both beans and beverage, as well as the quality of Conilon coffee (*C. canephora* Pierre ex A. Froehner). This represents a critical gap in our understanding that needs to be addressed. Furthermore, most biofortification studies have focused on the use of inorganic Se sources with rapid release, such as sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4) (Boldrin et al., 2013; Schiavon et al., 2020). However, recent findings have demonstrated the great potential of using the organic source Se-glycine, which is released more slowly and presents a lower risk of causing Se toxicity in plants (Li et al., 2023). Biofortification strategies should aim to increase Se concentration in coffee beans without causing plant toxicity or compromising crop yield. In this context, our hypothesis is that foliar Se application enhances the biofortification of beans and the Conilon coffee beverage, all while maintaining crop yield and sensory quality. Our objectives with this study were to evaluate the biofortification of Conilon coffee beans and beverages through the foliar application of different Se sources and rates by assessing their effects on the physicochemical attributes of the beans, yield, and beverage quality.

2 Materials and Methods

2.1 Experimental sites

The study was conducted during the 2023/2024 season in Linhares, Espírito Santo, Brazil, at two sites cultivated with Conilon coffee cv. Diamante Incaper 8112 (site 1: 19°18'01" S, 40°26'27" W; site 2: 19°18'18" S, 40°26'57" W) to assess the consistency of Conilon coffee's response to foliar Se application. The experimental sites were established in 2018 using 3.0 x 1.0 m spacing. Throughout the growing cycle, irrigation and fertilization were managed using a drip irrigation system, and plants were managed using Programmed Cycle Pruning (PCP) (Ferrão et al., 2019).

According to the Brazilian Soil Classification System (SiBICS) (Santos et al., 2018), the soils at both study sites are classified as Latossolo Vermelho-Amarelo (LVA) with a clayey texture. This classification corresponds to Xanthic Ferralsols in the World Reference Base for Soil Resources (WRB/FAO) (IUSS Working Group, 2022). Soil samples from both sites were collected from the 0.0–0.2 m depth layer. The physical and chemical characterization of the soil samples was performed following the methods described by Teixeira et al. (2017) (Table 1). According to the Köppen climate classification, the study region has an Aw climate (tropical wet with dry winter), with an average annual temperature of 22–24 °C and precipitation of 1,000–1,400 mm (Alvares et al., 2014).

2.2 Experimental design and treatments

Two experiments were conducted simultaneously using a randomized block design with a 2×5 factorial arrangement and four replications. Sodium selenate (Na_2SeO_4 – Se(VI)) and Se-bisglycinate (Se-Bis) were foliar-applied at five Se rates (0, 10, 20, 40, and 60 mg L^{-1}) during the small green fruit stage. The rates were determined based on results obtained by Mateus et al. (2021). Each experimental plot comprised rows of ten plants, with only the four central plants being evaluated. Foliar Se application was carried out in the morning with a motorized backpack sprayer under the following conditions: wind speeds of 3–10 km h^{-1}, relative humidity above 60%, and air temperature below 30 °C.

2.3 Harvest and post-harvest evaluations

The coffee was harvested manually through selective harvesting, selecting only the fruits in the cherry stage. Natural coffee was dried on a suspended terrace with continuous rotation until its moisture content reached 12% (Ferrão et al., 2019). After drying, the coffee was processed to obtain the green beans used in the evaluations described below.

2.3.1 Green bean production and sensory analysis

Green bean production (kg $plant^{-1}$) was measured by weighing green coffee samples on a high-precision electronic scale (0.0001 g). The sensory analysis of the coffee was conducted by five R-Graders, following the Coffee Quality Institute (CQI) Robusta Tasting Protocol (UCDA, 2010). Coffee samples were roasted 24h before sensory analysis. Roasting was done using the Laboratto model TGP-2 for approximately 10 min at 190 °C ± 10 °C. The roasting point of the samples was between the colors determined by the Agtron-SCA disks #65 and #55 for specialty coffees. The coffee samples were ground using a Bunn model G3 grinder with a medium/coarse grind. The infusion was made when the water reached between 92.2 and 94.4 ºC. The evaluations began when the temperature of the cups reached 55 ºC, respecting the 4-min time for tasting after the infusion. The attributes evaluated were fragrance, flavor, acidity, sweetness, mouthfeel, balance, aftertaste, uniformity, clean cup, and overall impression. The final score was obtained by summing the individual scores of each attribute, rated on a scale from 0 to 10.

2.3.2 pH, soluble solids, and titratable acidity

The pH, soluble solids (SS), and titratable acidity (TA) were determined according to the Association of Official Analytical Chemists (AOAC, 2023). The pH of the samples was measured using a digital pH meter (Kasvi, model K38-2014B), previously calibrated with pH 4 and 7 buffer solutions. Soluble solids (°Brix) were determined using a digital refractometer (Reichert Ar-200). Titratable acidity (mL NaOH 0.1 N 100 g^{-1}) was quantified by titration with sodium hydroxide (NaOH 0.1 N). The ratio between SS and TA (SS/TA) was calculated to characterize the balance between sweetness and acidity of the samples.

2.3.3 Proteins and phenolic compounds

The coffee beans' total protein content (g 100 g^{-1} DW) was determined according to the methodology described by the AOAC (2023). Total phenolic compounds (TPC; g GAE 100 g^{-1} DW) were determined using the methodology described by Zitha et al. (2022), with the Folin-Ciocalteu reagent. The TCP was quantified by spectrophotometry using a 96-well microplate reader (Biochrom EZ Read 2000).

2.3.4 Selenium concentration in coffee beans and beverage

Green coffee bean samples were dried in a forced-air oven at 65 ± 5 °C until a constant weight was achieved and ground in a stainless steel Willey knife mill. Roasted and ground coffee bean samples were prepared according to the procedures described for sensory analysis (UCDA, 2010). The coffee brew was prepared using a French press, with a ground coffee to high-purity deionized water ratio of 1:15 (w/v). The infusion was made once the water reached 93 ± 1 ºC. The mixture was allowed to rest for 4 min. Subsequently, the piston was pushed softly to the bottom of the press (Espitia-López et al., 2019; Santanatoglia et al., 2023). The digestion of green coffee beans (0.1 g), roasted coffee beans (0.1 g), and beverage (3 mL) samples was conducted following the USEPA 3051A protocol (USEPA, 2007). The process involved digestion with concentrated nitric acid (HNO_3) using a CEM® Mars-5 microwave system (CEM Corp, Matthews, NC) with pressure and temperature control. Selenium concentration was evaluated in green coffee beans (μg kg^{-1}), roasted coffee beans (μg kg^{-1}), and the coffee beverage (μg L^{-1}) using inductively coupled plasma-mass spectrometry (ICP-MS). A blank sample and a certified reference material (Peach Leaves – SRM 1547, National Institute of Standards and Technology – NIST, USA) were included in each analysis batch to ensure data reliability. The mean Se recovery rate was 97% (n = 4).

2.4 Statistical analysis

The data were tested for normality (Shapiro-Wilk) and homogeneity of variance (Bartlett) ($p \leq 0.05$). Analysis of variance (ANOVA) was performed using the F-test ($p \leq 0.05$). Interactions between factors (sources and rates) were evaluated, and means were compared using Tukey's test ($p \leq 0.05$). Principal Component Analysis (PCA) was applied to the dataset to identify patterns and correlations among variables. All statistical analyses were conducted using the R software (R Core Team, 2024).

3 Results

3.1 Green bean production and sensory analysis

Foliar Se application significantly increased the production of Conilon coffee at both sites (Fig. 1). At site 1, coffee yield showed a similar response to Se(VI) and Se-Bis applications. However, at site 2, the Se-Bis application resulted in the highest yield (3.24 kg plant^{-1}). Overall, Se applied at 20 mg L^{-1} promoted the highest coffee yield increments compared with the control (Figs. 1A and 1B).

Regarding sensory analysis, final scores similarly responded to both Se(VI) and Se-Bis applications at the evaluated sites. At site 1, foliar Se application rates did not significantly influence the final scores, which ranged from 79.60 to 82.25 points (Fig. 1C). Conversely, at site 2, foliar Se application at 60 mg L^{-1} significantly improved beverage quality compared with the control, achieving a final score of 81.10 points (Fig. 1D).

3.2 pH, soluble solids, titratable acidity, and SS/TA ratio

The pH, SS, TA, and SS/TA ratio of green coffee beans responded similarly to the application of Se(VI) and Se-Bis at the evaluated sites. At site 1, increasing foliar Se application rates did not significantly affect SS contents but decreased pH and the SS/TA ratio compared with the control. Furthermore, Se applied at 10 mg L^{-1} resulted in higher TA in green beans. At site 2, increasing foliar Se rates significantly decreased pH, SS, and the SS/TA ratio compared with the control. However, foliar Se application at 20 mg L^{-1} promoted higher TA in green beans (Table 2).

3.3 Proteins and phenolic compounds

Foliar Se application (sources and rates) did not significantly affect green coffee beans' protein and TCP contents at the evaluated sites (Table 2). Total protein content ranged from 12.39 to 14.37 g 100 g^{-1} DW at site 1 and 13.04 to 13.90 g 100 g^{-1} DW at site 2. TCP content, in turn, ranged from 4.00 to 4.18 g 100 g^{-1} DW at site 1 and 3.55 to 3.91 g 100 g^{-1} DW at site 2.

3.4 Selenium concentration in coffee beans and beverage

Selenium concentration in green coffee beans, roasted coffee beans, and coffee beverages responded similarly to Se(VI) and Se-Bis applications. Selenium was not detected in untreated plants. However, increasing foliar Se application rates significantly increased Se concentration in green coffee beans, roasted coffee beans, and beverages at the evaluated sites (Fig. 2). At site 1, foliar Se application at 60 mg L^{-1} resulted in the highest Se concentration in green coffee beans (620.70 µg kg^{-1}), roasted coffee beans (322.76 µg kg^{-1}), and coffee beverages (4.84 µg L^{-1}) compared with the control (Figs. 2A, 2C, and 2E). Similarly, at site 2, Se applied at 60 mg L^{-1} resulted in the highest Se concentration in green coffee beans (606.80

µg kg^{-1}), roasted coffee beans (427.87 µg kg^{-1}), and coffee beverages (5.29 µg L^{-1}) compared with the control (Figs. 2B, 2D, and 2F).

3.5 Principal Component Analysis

The PCA analysis showed that the first two principal components (PC1 and PC2) accounted for 67.30% and 67.80% of the total variability in the data from sites 1 and 2, respectively. At site 1, Se application rates were strongly correlated with Se concentration in green beans, roasted beans, and coffee beverages. Similarly, at site 2, Se application rates were strongly correlated with Se concentration in green beans, roasted beans, coffee beverages, and final score (Fig. 3).

4 Discussion

Our findings demonstrate that foliar Se application at 20 mg L^{-1} significantly enhanced Conilon coffee production compared with the control at both evaluated sites (Figs. 1A and 1B). This result aligns with the observations of Mateus et al. (2021), who reported a 38% increase in Arabica coffee yield following the foliar application of 20 mg Se L^{-1}. The positive effects of Se on coffee production can be attributed to its role in promoting plant physiological and biochemical processes when applied at optimal concentrations, as evidenced in previous studies (Chauhan et al., 2019; Feng et al., 2013; Lanza and Reis, 2021). Selenium plays a critical role in antioxidant metabolism, mitigating oxidative stress induced by reactive oxygen species (ROS) and enhancing plant tolerance to abiotic stresses such as extreme temperatures, drought, salinity, and heavy metal toxicity (Chauhan et al., 2019; Gupta and Gupta, 2017; Rengel et al., 2022). Furthermore, Se application at appropriate rates improves photosynthetic performance by protecting the antenna complex of the photosynthetic apparatus, thereby promoting crop growth and yield (Lanza and Reis, 2021; Yang et al., 2022). The action of these mechanisms likely explains the observed increase in Conilon coffee production following Se application.

Besides allowing the highest production, foliar Se application may influence the final score of coffee sensory analysis (Fig. 1). Sensory analysis remains the primary method to define coffee quality (Agnoletti et al., 2022). Coffee quality is closely related to the chemical composition of the roasted beans, which, in turn, is affected by the composition of the green beans and the post-harvest processing conditions (*e.g.*, handling, drying, storage, roasting, and grinding) (Franca and Oliveira, 2008). Furthermore, several factors influence the chemical composition of the beans and the sensory quality of the beverage throughout the coffee production chain, such as genetic factors, edaphoclimatic conditions, and crop management (Ahmed et al., 2021; Simmer et al., 2022). Our findings showed that foliar Se application at the small green fruit stage influences the beans' physicochemical attributes, including pH, SS, TA, and SS/TA ratio (Table 2), and may increase the quality of the Conilon coffee beverage (Fig. 1D).

Specialty coffees stand out for their unique sensory attributes, providing consumers with a superior-quality beverage (Silveira et al., 2016). To be classified as a specialty, the coffee must achieve a score ≥ 80 in sensory analysis (Guambi et al., 2024; Traore et al., 2018). At site 2, foliar Se application (60 mg L^{-1}) significantly improved beverage quality, achieving a final score of specialty coffee (81.10 points), surpassing the control value by 4.35 points (Fig. 1D). The PCA further corroborated these findings, revealing a strong positive correlation between Se application rates and the final score at site 2 (Fig. 3). While foliar Se application (sources and rates) did not result in significant differences in the final score at site 1, the coffee was still classified as specialty coffee, achieving an average score of 80.83 points (Fig. 1C).

The concentration of physicochemical compounds in green coffee beans plays a crucial role in determining the quality of the beverage after roasting (Hall et al., 2022). Among these compounds, SS is one of the most significant chemical properties associated with coffee quality (Sousa and Paiva, 2024). Soluble solids are primarily composed of sugars, caffeine, trigonelline, and chlorogenic acids (Nogueira and Trugo, 2003), and higher SS levels are known to enhance water solubility, thereby improving the body of the beverage. This characteristic is particularly valuable to the instant coffee industry (Junior et al., 2024). In this study, increasing Se application rates resulted in slightly reduced SS content at site 2. Despite this reduction, Conilon coffee plants treated with Se maintained SS values above 31 °Brix at both evaluated sites (Table 2). These findings align with those of Agnoletti et al. (2019), who reported that the species C. canephora naturally exhibits higher SS levels.

Acidity is another important sensory attribute significantly influencing coffee quality scoring (Anokye-Bempah et al., 2024). Small changes in the pH and TA can change the beverage's flavor profile and affect consumer preference (Batali et al., 2021). Coffee acidity is primarily determined by the type and concentration of acids present in the beans, including aliphatic acids (*e.g.*, acetic acid, citric acid, malic acid, and quinic acid) and chlorogenic acids (Yeager et al., 2023). The SS/TA ratio is also a critical parameter, as it is associated with the perception of sweetness in the beverage. An unbalanced SS/TA ratio can lead to undesirable sensory perceptions, such as a "diluted" or "too acidic" flavor (Junior et al., 2024). In this study, increasing foliar Se application rates reduced the pH and increased the TA of the coffee beans at both evaluated sites. The SS/TA ratio varied from 0.13 to 0.19, with the highest values observed in the control treatment at both sites (Table 2). These findings suggest that foliar Se application may influence the acid profile of coffee beans, potentially changing the sensory balance of the beverage. While these results provide insights into the effects of Se on coffee acidity, further research is needed to elucidate the underlying mechanisms by which Se changes acid profiles in green coffee beans.

The protein and TPC contents in green coffee beans were unaffected by foliar Se application, regardless of the sources and rates applied, at both evaluated sites. Protein content ranged from 12.39 to 14.37% (Table 2), consistent with the range from 11 to 17% reported by Franca and Oliveira (2008). Total phenolic compounds content in *C. canephora* beans typically range from 7 to 14.4% (Hall et al., 2022), but lower values ranging from 4.03 to 6.80% were reported by Agnoletti et al. (2019). Our findings showed that TPC content ranged from 3.55 to 4.18% (Table 2). Total phenolic compounds are predominantly composed of chlorogenic acids (CGA), which are known for their antioxidant properties and their contribution to the astringency, bitterness, and final acidity of the coffee beverage (Farah and Donangelo, 2006; Yeager et al., 2023). Higher levels of CGA have been associated with lower sensory quality (Agnoletti et al., 2019; Farah et al., 2006). Therefore, the lower TPC values observed in this study may be linked to a final score exceeding 80 points (specialty coffee).

Applying Se(VI) and Se-Bis resulted in similar effects on the Se concentration in Conilon coffee, including green and roasted beans and their respective beverages. On the other hand, foliar Se application at 60 mg L^{-1} significantly increased Se concentration in coffee beans and beverages compared with the control at both evaluated sites. At this rate, Se concentration reached 620.70 and 606.80 µg kg^{-1} in green coffee beans, 322.76 and 427.87 µg kg^{-1} in roasted coffee beans, and 4.84 and 5.29 µg L^{-1} in coffee beverages at sites 1 and 2, respectively (Fig. 2). These findings are supported by PCA, which revealed a strong positive correlation between the applied Se rates and Se concentration in coffee beans and beverages at both evaluated sites (Fig. 3).

For Arabica coffee, foliar Se application (0 to 160 mg L^{-1}) using sodium selenate as the source significantly increased Se concentration in green coffee beans, ranging from 0.116 to 4.47 mg kg^{-1} (Mateus et al., 2021). However, the authors stated that foliar Se application as sodium selenate at rates above 100 mg L^{-1} is not recommended for coffee plants due to chlorophyll oxidation. In this study, no visual symptoms of toxicity were observed in plants treated with Se(VI) and Se-Bis. In addition, the highest Conilon coffee production results were achieved by applying low Se rates (20 mg L^{-1}) (Figs. 1A and 1B). These findings highlight the importance of Se application in enhancing Se concentration in coffee beans. However, caution is necessary regarding the Se source and rate to ensure effective biofortification without compromising coffee yield.

Foliar Se application at the small green fruit stage effectively enhanced Se concentration in green coffee beans at the evaluated sites. However, significant losses of Se were observed during the roasting and brewing processes. Roasting reduced Se concentration in roasted beans by 48.00% and 38.92% at sites 1 and 2, respectively, compared to green beans. These losses may be related to Se volatilization during roasting due to high temperatures (190 °C ± 10 °C). Many organic Se compounds are relatively volatile, and losses by volatilization may occur at temperatures above 120 °C (Olivas et al., 1994; Smrkolj and Stibilj, 2004). Furthermore, 98.54% and 99.00% of the Se present in roasted beans were not extracted into the beverage at sites 1 and 2, respectively (Fig. 2). Our findings indicate that roasting considerably reduces Se concentration and most remaining Se in roasted beans is not transferred to the beverage, likely due to low extraction efficiency during brewing. Considering the higher Se concentrations in the beverage obtained in this study (4.84 and 5.29 μg L^{-1}) (Figs. 2E and 2F) and daily coffee consumption of 3 to 4 cups (1 cup = 150 mL) (Poole et al., 2017), the estimated Se intake by humans (for 3 and 4 cups of coffee, respectively) should be 2.18 and 2.90 μg day^{-1} at site 1 and 2.38 and 3.17 μg day^{-1} at site 2. These results demonstrate that foliar Se application at 60 mg L^{-1} resulted in an estimated intake ranging from 3.11 to 4.52% of the recommended daily Se intake for adults (70 μg day^{-1}), as reported by Kipp et al. (2015). The contribution of Se biofortified coffee for Se intake can be more significant if the causes behind the low extraction efficiency during brewing are elucidated.

To the best of our knowledge, no published studies have investigated the effects of the foliar application of Se-Bis on biofortification and the quality of Conilon coffee. In this study, most evaluated variables exhibited similar responses to both Se-Bis and Se(VI) applications, except for the yield at site 2, which was higher with the Se-Bis application (Fig. 1B). These results highlight the significant potential of Se-Bis as an organic Se source for Conilon coffee biofortification. Sustainable plant nutrition is driven by the core concept of 4R Nutrient Stewardship (applying the Right Source, at the Right Rate, at the Right Time, and in the Right Place) (Johnston and Bruulsema, 2014). Our findings suggest that foliar Se application (Right Place) at 60 mg L^{-1} (Right Rate), using Se-Bis and Se(VI) (Right Source) at the small green fruit stage (Right Time), is a promising strategy to enhance the sensory and nutritional quality of Conilon coffee and contribute to Se intake in the human diet without causing toxicity to plants or compromising crop yield.

5 Conclusion

Foliar Se application at 60 mg L^{-1} using Se-Bis and Se(VI) during the small green fruit stage may enhance Conilon coffee quality and increase Se dietary intake without compromising coffee yield compared to untreated plants. However, further studies are required to understand better how Se application influences the concentration of physicochemical compounds (*e.g.*, pH, SS, and TA) in green coffee beans and their subsequent impact on beverage quality. Applying Se at rates up to 60 mg L^{-1} significantly increased Se concentration in both green and roasted coffee beans, contributing to an estimated Se intake of 3.11 to 4.52% of the recommended daily intake for adults, based on the consumption of 3–4 cups (150 mL) of coffee per day. However, significant Se losses occurred during roasting (38.92–48.00%) and brewing (98.54–99.00%) relative to green and roasted coffee beans, respectively. Furthermore, applying Se at rates up to 60 mg L^{-1} using Se(VI) and Se-Bis as sources proved effective in supplying Se to Conilon coffee without inducing visual Se toxicity symptoms in plants.

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Declaration of Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Authors' contributions

All authors contributed intellectual input and assistance to this study and manuscript preparation.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Table 1 Physical and chemical characterization of soils at the study sites in Linhares, Espírito Santo, Brazil.

Sites	Depth (m)	Physical characterization	Chemical characterization
Site 1	0.0–0.2	Sand = 400 g kg^{-1}; silt = 70 g kg^{-1}; clay = 530 g kg^{-1}	OM = 3.53 dag kg^{-1}; pH = 6.3; Ca = 5.73 $cmol_c$ dm^{-3}; Mg = 1.94 $cmol_c$ dm^{-3}; K = 130.78 mg dm^{-3}; Al^{3+} = 0.20 $cmol_c$ dm^{-3}; H+Al = 3.10 $cmol_c$ dm^{-3}; SB = 8.01 $cmol_c$ dm^{-3}; CEC = 11.11 $cmol_c$ dm^{-3}; BS = 72.06%; P = 198.03 mg dm^{-3}; S-SO_4^{2-} = 11.7 mg dm^{-3}; Zn = 12.1 mg dm^{-3}; Fe = 72.6 mg dm^{-3}; Mn = 14.8 mg dm^{-3}; Cu = 1.64 mg dm^{-3}; B = 0.4 mg dm^{-3}
Site 2	0.0–0.2	Sand = 410 g kg^{-1}; silt = 40 g kg^{-1}; clay = 550 g kg^{-1}	OM = 4.13 dag kg^{-1}; pH = 6.6; Ca = 6.75 $cmol_c$ dm^{-3}; Mg = 1.44 $cmol_c$ dm^{-3}; K = 64.57 mg dm^{-3}; Al^{3+} = 0.20 $cmol_c$ dm^{-3}; H+Al = 2.00 $cmol_c$ dm^{-3}; SB = 8.36 $cmol_c$ dm^{-3}; CEC = 10.36 $cmol_c$ dm^{-3}; BS = 80.65%; P = 13.47 mg dm^{-3}; S-SO_4^{2-} = 25.90 mg dm^{-3}; Zn = 14 mg dm^{-3}; Fe = 102.6 mg dm^{-3}; Mn = 10.6 mg dm^{-3}; Cu = 13.99 mg dm^{-3}; B = 0.22 mg dm^{-3}

Organic matter (OM) (wet oxidation with $Na_2Cr_2O_7$ 4 N + H_2SO_4 10 N); pH (H_2O); sum of bases (SB); cation exchange capacity at pH 7 (CEC); base saturation (BS); P, K, Fe, Zn, Mn, and Cu (extractant Mehlich-1); Ca, Mg, and Al^{3+} (extractant KCl 1 mol L^{-1}); H+Al (extractant SMP); B (extractant hot water); S-SO_4^{2-} (extractant monocalcium phosphate).

Table 2 Mean and standard deviation of pH, soluble solids (SS, °Brix), titratable acidity (TA, mL NaOH 0.1 N 100 g^{-1}), SS/TA ratio, protein (g 100 g^{-1} DW), and total phenolic compounds (TPC, g GAE 100 g^{-1} DW) in green coffee beans in response to foliar application of Se(VI) and Se-Bis at different Se rates (mg L^{-1}) in Conilon coffee (*C. canephora* cv. Diamante Incaper 8112) cultivated at sites 1 and 2 in Linhares, Espírito Santo, Brazil.

Site 1						
Sources	pH	SS	TA	SS/TA	Protein	TPC
Se-Bis	5.34 ± 0.03 a	32.13 ± 0.99 a	200.67 ± 20.86 a	0.16 ± 0.02 a	13.21 ± 0.89 a	4.10 ± 0.20 a
Se(VI)	5.35 ± 0.03 a	32.40 ± 1.12 a	200.00 ± 20.62 a	0.16 ± 0.02 a	13.29 ± 0.66 a	4.01 ± 0.26 a
Rates	pH	SS	TA	SS/TA	Protein	TPC
0	5.38 ± 0.03 a	32.00 ± 1.55 a	166.67 ± 5.16 c	0.19 ± 0.01 a	14.37 ± 0.49 a	4.00 ± 0.21 a
10	5.36 ± 0.01 ab	32.67 ± 1.21 a	222.50 ± 9.87 a	0.15 ± 0.01 b	13.37 ± 0.19 a	4.02 ± 0.08 a
20	5.33 ± 0.01 b	32.83 ± 0.98 a	205.00 ± 7.75 b	0.16 ± 0.01 b	12.91 ± 0.61 a	4.05 ± 0.20 a
40	5.32 ± 0.02 b	32.17 ± 0.41 a	203.33 ± 13.66 b	0.16 ± 0.01 b	12.39 ± 0.30 a	4.04 ± 0.41 a
60	5.33 ± 0.03 b	31.67 ± 0.52 a	204.17 ± 5.85 b	0.15 ± 0.01 b	13.19 ± 0.42 a	4.18 ± 0.15 a
Site 2						
Sources	pH	SS	TA	SS/TA	Protein	TPC
Se-Bis	5.28 ± 0.07 a	32.33 ± 1.23 a	247.67 ± 24.27 a	0.13 ± 0.02 a	13.75 ± 0.67 a	3.74 ± 0.27 a
Se(VI)	5.30 ± 0.06 a	32.87 ± 0.99 a	235.67 ± 26.11 a	0.14 ± 0.02 a	13.36 ± 0.52 a	3.70 ± 0.33 a
Rates	pH	SS	TA	SS/TA	Protein	TPC
0	5.36 ± 0.04 a	33.67 ± 0.52 a	213.33 ± 10.33 c	0.16 ± 0.01 a	13.04 ± 0.28 a	3.78 ± 0.34 a
10	5.23 ± 0.09 b	31.83 ± 1.72 b	241.67 ± 44.01 b	0.14 ± 0.02 b	13.58 ± 0.70 a	3.74 ± 0.37 a
20	5.29 ± 0.06 b	32.83 ± 0.98 b	262.50 ± 9.87 a	0.13 ± 0.01 b	13.90 ± 0.55 a	3.55 ± 0.16 a
40	5.28 ± 0.03 b	32.50 ± 0.55 b	249.17 ± 4.92 b	0.13 ± 0.01 b	13.85 ± 0.77 a	3.91 ± 0.10 a
60	5.30 ± 0.04 b	32.17 ± 0.75 b	241.67 ± 7.53 b	0.13 ± 0.01 b	13.40 ± 0.44 a	3.61 ± 0.35 a

Means followed by the same letter within a column are not significantly different according to Tukey's test ($p \leq 0.05$).

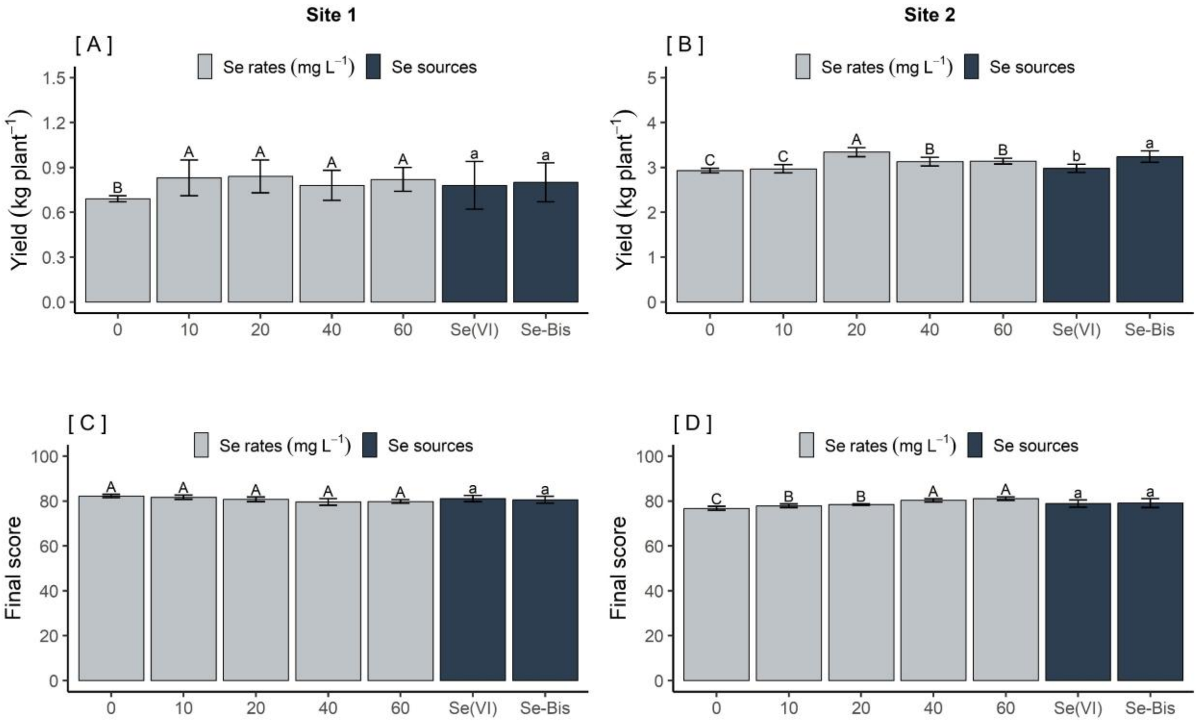

Fig. 1 Coffee bean yield (A and B) and final score (C and D) in response to foliar application of Se(VI) and Se-Bis at different Se rates in Conilon coffee (*C. canephora* cv. Diamante Incaper 8112) cultivated at sites 1 and 2 in Linhares, Espírito Santo, Brazil. Means followed by the same lowercase letter (comparing sources) or uppercase letter (comparing rates) are not significantly different according to Tukey's test ($p \leq 0.05$).

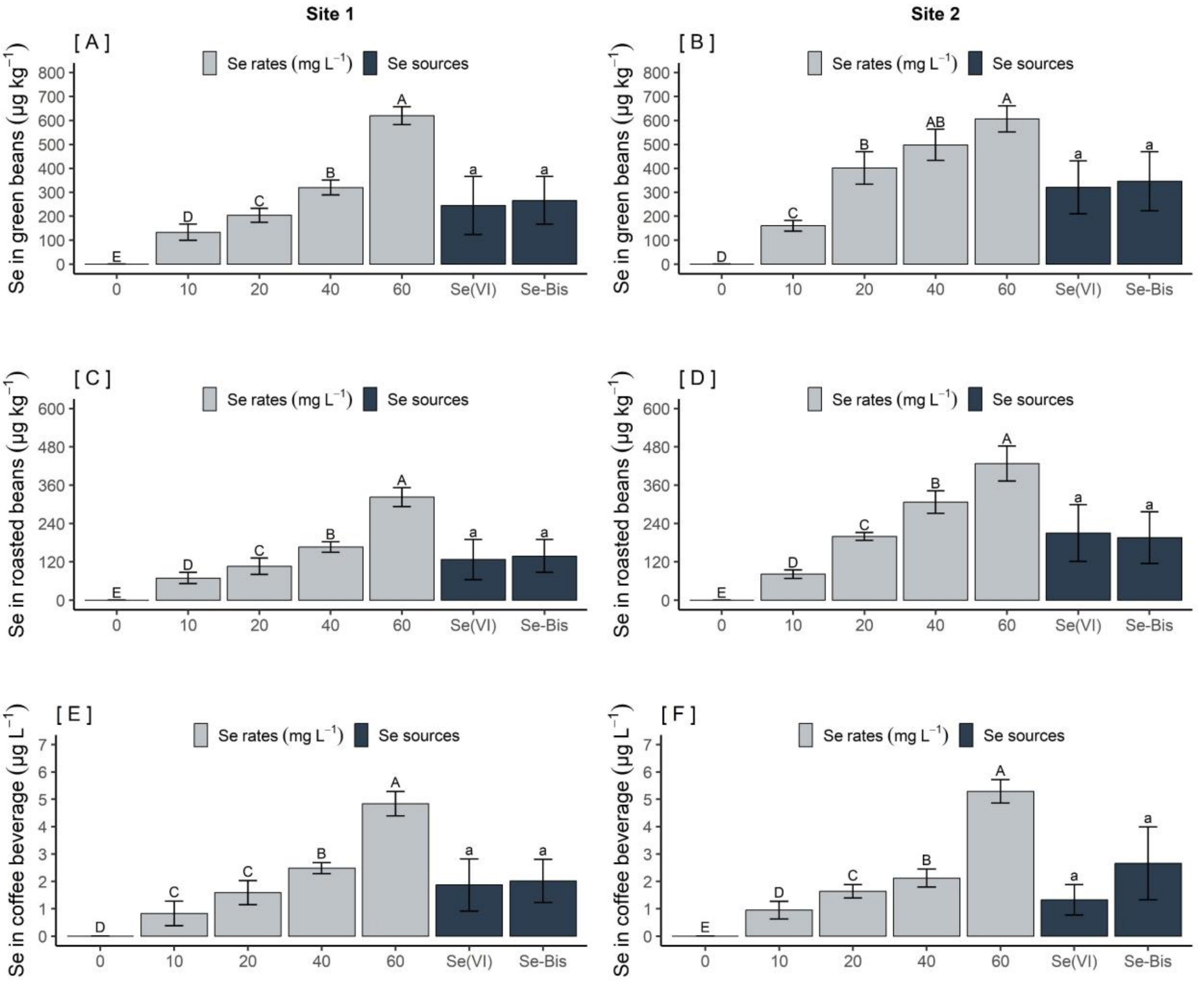

Fig. 2 Selenium concentration in green (A and B) and roasted (C and D) coffee beans and in the beverage (E and F) in response to foliar application of Se(VI) and Se-Bis at different Se rates in Conilon coffee (*C. canephora* cv. Diamante Incaper 8112) cultivated at sites 1 and 2 in Linhares, Espírito Santo, Brazil. Means followed by the same lowercase letter (comparing sources) or uppercase letter (comparing rates) are not significantly different according to Tukey's test ($p \leq 0.05$).

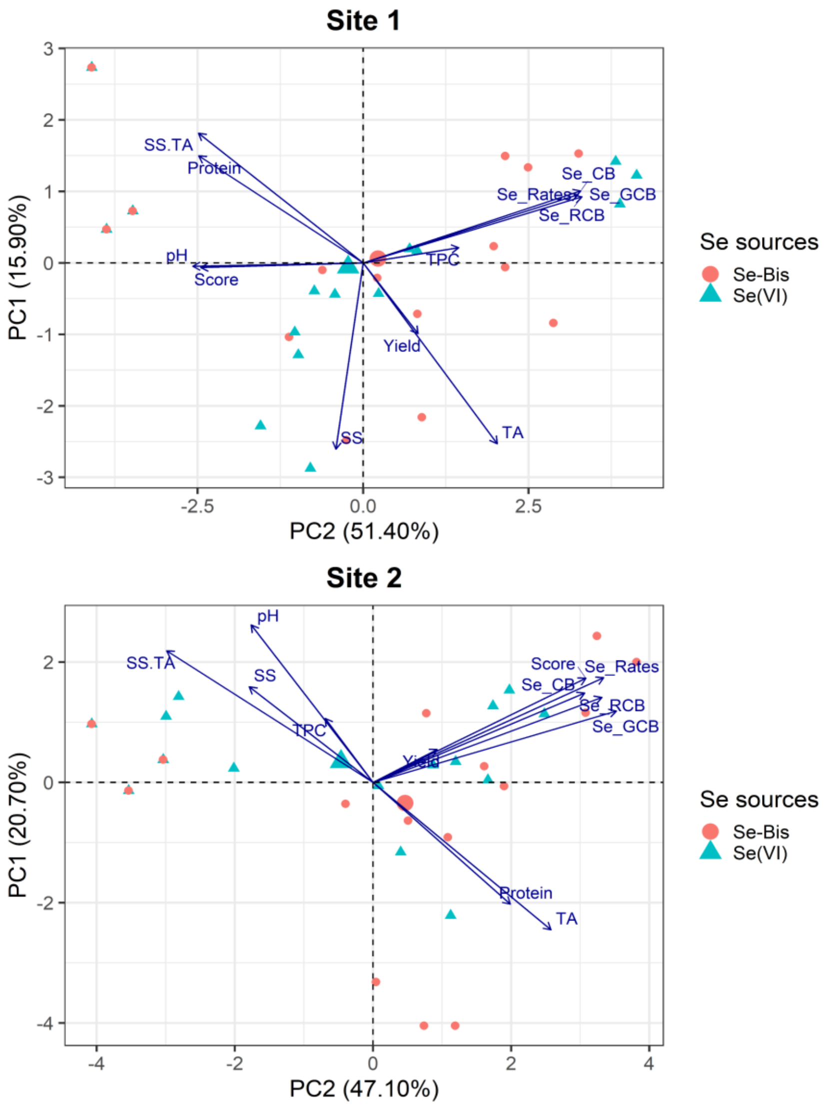

Fig. 3 Principal component analysis of Se rates (Se_Rates), Se concentration in green coffee beans (Se_GCB), Se concentration in roasted coffee beans (Se_RCB), Se concentration in the coffee beverage (Se_CB), pH (pH), soluble solids (SS), titratable acidity (TA), SS/TA ratio (SS.TA), protein (Protein), total phenolic compounds (TPC), grain yield (Yield), and final score (Score) in response to foliar application of Se(VI) and Se-Bis at different Se rates in Conilon coffee (*C. canephora* cv. Diamante Incaper 8112) cultivated at sites 1 and 2 in Linhares, Espírito Santo, Brazil.

THIRD PART

2 FINAL REMARKS

This thesis provides robust evidence that foliar Se application—carried out at the small green fruit stage using either a mineral (Se(VI)) or organic (Se-Bis) source—is an effective strategy to ensure the yield of Conilon coffee under adverse climatic conditions, while also enhancing coffee quality in both sensory and nutritional aspects. In chapter 1, it was demonstrated that Se application optimized the antioxidant metabolism of Conilon coffee plants, reduced oxidative damage, and improved photosynthetic performance. As a result, coffee yield increased by up to 21.60%, without inducing toxicity or disrupting the nutritional balance of the plants. Chapter 2 showed that foliar Se application promotes the biofortification of both coffee beans and the brewed beverage. Although significant Se losses were observed during roasting and brewing, the final beverage remained a supplementary dietary source of Se. Additionally, Se application positively influenced the sensory quality of the beverage, with cupping scores exceeding 80 points (specialty coffee threshold). Future research should focus on: (i) evaluating Se application in other cultivars and under different edaphoclimatic conditions; (ii) testing Se application at different phenological stages; (iii) investigating the combined application of Se with other nutrients involved in anti-stress pathways (*e.g.*, Zn, Cu, Mn, Mg, and Fe); (iv) evaluating Se application through fertigation; and (v) developing strategies to minimize Se losses during roasting and brewing, including exploring new coffee preparation methods.

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