



Improving growth, production and oil quality of two olive cultivars (Manzanillo and Koroneiki) using calcium oxide in the form of nanoparticles

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ABSTRACT

In every country of the globe, and particularly in the Mediterranean area, olives are considered one of the most significant fruit crops. Calcium fertilization is a common agricultural practice in olive orchards to sustain a good fruit set and development thus, an abundant yield with high quality standards will be expected. Calcium nanoparticles application has emerged as an efficient strategy for supplying plants with their calcium needs owing to their enhanced characteristics as a result of their high surface-to-volume ratio. The purpose of this research was to evaluate how calcium oxide nanoparticles CaO NPs will affect the growth, yield and fruit quality parameters of Manzanillo and Koroneiki olive cultivars grown in a commercial orchard in Beheira governorate, Egypt. The study was conducted for 2 consecutive seasons (2022 and 2023). Trees were sprayed with CaO NPs in 3 different concentrations of (100, 200, and 300) ppm in March, April, and May (before blooming, during full bloom, and after fruit set). The water-sprayed trees were considered as control. Results showed a significant improvement in almost all the studied parameters including flowering, fruit set, yield and oil content with the application of CaO NPs to olive trees especially the 200 ppm and 300 ppm concentrations.

Keywords: olive, calcium oxide, Nano fertilization, fruit quality, oil percentage, oil

1. Introduction

A major horticultural crop widely cultivated in the Mediterranean region is the olive (*Olea europaea*). The global demand for olive oil is increasing due to its superior sensory qualities, nutritional value, and therapeutic benefits. The health advantages of "virgin olive oil" are largely attributed to its fatty acids and various metabolites, including sugars, phenolic compounds, and chlorophyll (Franco *et al.*, 2014). Diets rich in olive oil have been shown to reduce the occurrence of cardiovascular diseases, cancer, and oxidative stress (Knoops *et al.*, 2004).

The synthesis of nanoparticles has garnered significant interest due to their numerous advantageous properties and applications across various industries. While physical and chemical methods for nanoparticle synthesis are becoming increasingly recognized, the biosynthesis of nanoparticles using diverse plant extracts offers a more sustainable and eco-friendly alternative. This green synthesis approach presents an opportunity for researchers worldwide to investigate the potential of different plants in nanoparticle production (Savithamma *et al.*, 2016). For an extended period, scientists have been developing innovative methods for creating nanoparticles. In fact, compared to physical, chemical, or microbiological synthesis, the green method is notably straightforward, efficient, and environmentally safe. Chemical synthesis often involves hazardous solvents and requires significant energy, with high-temperature processes, making it less ideal. as well as an organism included combination, isn't realistic in today's world. Given that choosing to synthesise nanoparticles using green

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synthesis is the best option (Anamika *et al.*, 2012). The beneficial features and quality of oil are influenced by various factors, such as the kind of cultivar, agroclimatic conditions, and orchard management strategies (Tura *et al.*, 2007). The nutritional condition of olive trees in orchard management, as well as the amount and quality of their oil, are determined by the type and quantity of fertilizers used for better development and yield (Sayyad-amin *et al.*, 2015; Bouhafa *et al.*, 2014). Generally speaking, calcium is a basic substance present in the earth. It is typically found in the mineral forms of calcite, dolomite, and gypsum in sedimentary rocks. In terms of mass, it is the fifth most plentiful element. Calcium performs a variety of compound exercises, acts as a cofactor to activate various types of enzymes, engages in membrane transport metabolisms, takes up nitrate (a useful form of nitrogen), increases biomass proportion, and speeds up photosynthetic rate, all of which are necessary for plants to grow and thrive (Savithramma, 2002; Savithramma *et al.*, 2007). It has been demonstrated that Ca^{2+} improves salinity and promotes plant development (Savithramma & Swamy, 1995; Kedarnath Reddy & Savithramma, 2013). Calcium is found in over 80 distinct compounds, often known as calcium salts. Lime, or calcium carbonate, is one such substance. A particular variety of calcium carbonate is a Calcium carbonate is an essential part of nursery lime, sometimes referred to as agricultural lime. The purpose of nano-fertilizers is to increase the pH and water content of the soil; they are less dangerous and more useful than other agricultural inputs. Recent research has focused on the potential of nanotechnology for the nutritional value, quality, and safety of food production, according to (Thiruvengadam *et al.*, 2018). Developments in nanoscience have enabled the development of fertilizer formulations for enhanced cellular penetration and increased nutrient uptake efficiency (Suppan, 2013). Research indicates that applying nanofertilizers to some fruit tree species might boost crop productivity and enhance the fruit is chemical and physical properties (Zagzog and Gad 2017). There have been reports that pomegranate trees that have received nano-fertilizers including calcium, boron, and calcium have produced more fruit and had higher levels of mineral elements in their leaves (Davarpanah *et al.*, 2016, 2017, 2018). The beneficial effects of nano-boron fertilizer foliar spray on olive oil quality, including total phenolic content and antioxidant capacity, have been shown in earlier research (Rohi Vishekaii *et al.*, 2019). To the best of our knowledge, however, no research has been done to assess the impacts on olive oil of standard calcium fertilizer sources with nano-chelated calcium. features of quality. This study aimed to assess the impact of urea and nano-calcium fertilizer on fruit output and quality indicators related to olive oil.

2. Material and Methods

2.1. Plant material and experimental site

Plant material and experimental site: this study was conducted for the two consecutive years 2022 and 2023 on a commercial olive orchard located in Beheira Governorate, Egypt (latitude 30.61°N, longitude 30.43°E) using Manzanillo and Koroneiki olive cultivars. The investigated 15-year-old olive trees were grown 4x6 m apart in loamy sand soil with pH of 7.7 and EC of 1.6 dS/m (Table 1). Healthy trees of similar vigor and appearance were chosen, trees were subjected to a drip irrigation system and received identical horticultural practices.

Table 1: Soil physical and chemical properties of the experimented site

Physical analysis		Chemical analysis			
		Cations (meq/l)		Anions (meq/l)	
		Ca ⁺⁺	8.7	CO ₃ ⁻	Zero
Sand	84.2%	Mg ⁺⁺	4.0	HCO ₃ ⁻	0.52
Silt	11.8%	Na ⁺	2.3	Cl ⁻	11.48
Clay	4.1 %	K ⁺	1.0	SO ₄ ⁻⁻	4.0
Texture class:	Loamy sand				
Soil pH	7.7	Available N: 0.78%			
E.C (dS/m)	1.6	Available P: 0.32%			
Organic matter	3.54 %	Available K: 0.46%			

2.2. Calcium oxide nanoparticles (CaO NPs) preparation

CaO nanoparticles were prepared as described by Gandhi *et al.* (2021) with little modification using calcium chloride (CaCl₂) solution and *Ocimum tenuiflorum* leaf extract as raw materials, 95 mL of 0.1 M CaCl₂ aqueous solution, and 5 mL of *Ocimum tenuiflorum* leaf extract were mixed with continuous stirring. The consequent solution was centrifuged at 12000 rpm for 15 min. The supernatant was discarded and CaO NPs pellets were washed with distilled water and dried overnight at room temperature. The characterization of nanoparticles was performed by UV–visible spectroscopy (Hitachi U2900) within the range of 200–700 nm and the size of CaO NPs was analyzed using the Beta size analyzer (BT 90).

2.3. Treatments

The study included three CaO NPs concentrations plus the control treatment (four treatments with three replicates each, and one tree per replicate). Fertilization with CaO NPs was used as foliar application with 100 ppm (T1), 200 ppm (T2), and 300 ppm (T3). Spraying with water was considered as control. Treatments were applied in three different foliar sprays in March, April, and May (before blooming, during full bloom, and after fruit set). Twenty of the one-year-old shoots well distributed around the tree canopy were carefully selected and labeled for measuring the investigated parameters. These shoots were at a height of 150 cm above ground. The chosen trees were arranged in a completely randomized design.

2.4. Analysis of oil quantity and quality

Using hexane at a boiling temperature of 60 to 80 °C, the traditional Soxhelt method was used to determine the oil content of fruit pulp. As a proportion of the dry pulp's weight, the results were presented (Avidan *et al.*, 1997). The examination of acidity (% of oleic acid), peroxide value (meq O₂ kg⁻¹), and UV absorbance (K232 and K270) was conducted in accordance with EEC 2568/91 (1991). The carotenoid and chlorophyll levels of the oil were determined at 470 and 670 nm, respectively, using a spectrophotometer (Isabel Minguez-Mosquera *et al.*, 1991). After the fruits reached the green ripening stage, they were taken out of each replicate and their total phenolic content (TPC) was calculated by weighing them. Crop production efficiency was calculated by dividing the yield of each tree by the area of its trunk cross-section (Rosati *et al.*, 2017).

2.5. Statistical analysis

The recorded data were statistically processed using the analysis of variance (ANOVA). The means were compared using Duncan's multiple range test and the Least Significant Difference (LSD) at the level of probability $p \leq 0.05\%$.

3. Results

3.1. Leaf mineral content

Leaf mineral content: Data presented in Table 2 indicates that leaf mineral content of the investigated olive cultivar has been positively affected by CaO NPs fertilizer application. Most of the applied concentrations showed significant increases in leaf nutrient content of the treated trees compared with the control in both Manzanillo and Koroneiki olive cultivars.

The maximum leaf N contents of 1.77% and 1.68% were recorded in trees treated with 300 ppm CaO NPs in Koroneiki and Manzanillo olive cultivars respectively. The remarkable increase in leaf Ca content (1.4 and 1.3 times higher than the control) in Koroneiki and Manzanillo olive cultivars respectively was also observed in treated olive trees with the highest CaO NPs concentration. Similar results were documented by Vishekaii *et al.* (2019) and Genaidy *et al.* (2020) applying nutrients in the form of nanoparticles on Zard and Picual olive cultivars.

Table 2: Effect of Calcium oxide nanoparticles (CaO NPs) using foliar application on leaf mineral content of Manzanillo and Koroneiki olive cultivars. Data presented are means of the two seasons

CaO NPs	N %	P %	K %	Ca %	B (ppm)	Fe (ppm)
Manzanillo						
Control	1.31 c	0.13 b	1.16 b	1.39 c	27.68 b	123.61 bc
100 ppm	1.53 b	0.13 b	1.31 ab	1.52 b	34.31ab	142.17 b
200 ppm	1.61 ab	0.14 a	1.35 ab	1.76 ab	36.43 a	151.91 a
300 ppm	1.68 a	0.14 a	1.47 a	1.87 a	39.62 a	153.36 a
Koroneiki						
Control	1.41 c	0.13 c	1.15 c	1.35 d	23.27 b	128.35 b
100 ppm	1.62 b	0.14 b	1.35 ab	1.48 c	34.65 ab	139.46 b
200 ppm	1.59 b	0.14 b	1.58 a	1.65 b	38.86 ab	145.37 ab
300 ppm	1.77 a	0.15 a	1.58 a	1.89 a	43.63 a	153.61 a

3. 2. Effect of CaO NPs on flowering aspects of olive trees:

The number of inflorescences per shoot of Manzanillo olive cultivar ranged from 10.12 to 13.04 with the highest number recorded during the 2023 season on trees sprayed with 300 ppm of CaO NPs (Table 3). The same pattern was observed with Koroneiki olive trees recording 15.14 as the highest number of inflorescences per shoot. There was an increase in the number of total flowers per inflorescence as a result of applying CaO NPs to the studied cultivars. This increase was significant for Manzanillo olives during 2022 using any of the investigated concentrations while being significant during 2023 only after using the 200 ppm and 300 ppm concentrations. Concerning Koroneiki olive trees, the significant increase in the number of total flowers per inflorescence was observed only with the concentrations of 200 ppm and 300 ppm in both seasons. As for the inflorescence length, the significant increase was recorded only on treated Koroneiki olive trees during 2023. Similar results of flowering characteristics improvement were reported by ElHady *et al.* (2020) when studying the effect of foliar spray with some calcium fertilizers on the flowering of Kalamata and Manzanillo olive cultivars.

Table 3: Effect of Calcium oxide nanoparticles (CaO NPs) using foliar application on flowering of Manzanillo and Koroneiki olive cultivars, data presented are of the two seasons (2022 and 2023).

CaO NPs	No. of inflorescences /shoot		No. of total flowers/inflorescence		Inflorescence length (cm)	
	2022	2023	2022	2023	2022	2023
Manzanillo						
Control	10.23b	9.35b	20.81b	10.32b	2.74a	2.70a
100 ppm	10.12b	12.73a	22.94a	11.64ab	2.66a	2.79a
200 ppm	11.47ab	11.80ab	23.07a	13.81a	2.81a	2.86a
300 ppm	12.62a	13.04a	24.16a	15.12a	2.84a	2.97a
Koroneiki						
Control	11.68b	10.31b	18.67c	11.37b	2.90a	2.82c
100 ppm	12.47ab	13.54ab	18.30c	12.00b	3.04a	2.98b
200 ppm	13.22a	14.29ab	20.41b	14.62a	3.12a	3.00b
300 ppm	13.86a	15.14a	22.94a	14.84a	3.21a	3.19a

3. 3. Effect of CaO NPs on fruit set, yield, and oil content:

The foliar application of CaO NPs solutions on the investigated olive trees has considerably improved the studied parameters. The initial fruit set of Manzanillo olive cultivar has been enhanced on the treated trees compared to the control ones both in on- and off-years, recording an increase of 45%

in 2022 and 57% in 2023 when using the highest concentration of CaO NPs (300 ppm), a similar pattern was observed with koroneiki olive cultivar (Table 4). The final fruit set ranged from 18.14% to 24.31% and from 7.34% to 9.63% on Manzanillo and Koroneiki olive trees respectively during 2022, showing a significant increase in the percentage of final fruit set with almost all the investigated concentrations of CaO NPs over the control, the pattern during 2023 was much alike with a bit increase in both cultivars. The positive effect of calcium foliar sprays on pollen germination and development in various fruit crops has been documented by Bons and Sharma (2023) which consequently will be translated into a higher percentage of fruit set.

Yields (Kg/tree) of Manzanillo olives during the on-year (2022) have ranged from 28.36 Kg/tree from the untreated trees to 35.69 Kg/tree from the trees sprayed with 300 ppm CaO NPs recording an increase of 26% over the control, the other two concentrations also showed an increase over the control which was significant only with the concentration 200 ppm. During the off-year (2023), a significant yield increase was recorded with all three studied concentrations. Regarding yields of Koroneiki olives, they were generally higher in comparison to those of Manzanillo olives, ranging from 32.38 Kg/tree from the untreated trees to 46.56 Kg/tree from the trees sprayed with 300 ppm CaO NPs recording an increase of 44% over the control. Similar results were reported by El-Sharkawy (2014) studying the effect of foliar calcium sprays in the form of calcium chloride on the productivity of Kalamata olive cultivar. Also, in agreement with our results, foliar sprays of chelated Ca or calcium chloride on Manzanillo and Kalamata olive cultivars have caused a significant increase in initial and final fruit sets as well as yields (El-Hady *et al.*, 2020).

Table 4: Effect of Calcium oxide nanoparticles (CaO NPs) using foliar application on fruit set, yield, and oil content % (on dry weight basis) of Manzanillo and Koroneiki olive cultivars, data presented are of the two seasons (2022 and 2023).

CaO NPs	Initial fruit set (%)		Final fruit set (%)		Yield (kg/tree)		% Oil (on dry weight basis)	
	2022	2023	2022	2023	2022	2023	2022	2023
Manzanillo								
Control	31.34c	37.58d	18.14c	21.76c	28.36c	22.63c	43.55b	43.27b
100 ppm	34.02c	42.67c	19.30bc	23.75b	28.75c	24.61b	44.14 b	46.35 ab
200 ppm	40.36b	51.20b	20.12b	26.84a	32.41b	28.47a	47.55 ab	46.88 ab
300 ppm	45.64a	59.32a	24.31a	28.42a	35.69a	29.82a	49.43 a	48.23 a
Koroneiki								
Control	11.48b	13.36b	7.34c	8.23c	32.38c	28.64c	44.71b	45.36b
100 ppm	12.23ab	14.68b	8.32b	9.14b	37.25b	34.78b	47.81 ab	47.14 b
200 ppm	14.81a	14.34b	8.51b	10.02a	45.19ab	39.56ab	53.49 a	53.48 a
300 ppm	15.45a	17.30a	9.63a	10.85a	46.56a	40.23a	51.92 a	53.72 a

3. 4. Effect of CaO NPs on oil quality parameters:

The quality parameters of olive oil from treated olive trees are shown in Table 5. The content of free fatty acids of the oil was reduced by calcium fertilizers. The lowest value of this parameter was obtained in the oil with the application of Nano 200 and 300 ppm. The value of peroxide showed a significant response to CaO NPs at concentrations of 200 ppm and 300 ppm.

Chlorophyll and carotenoid pigments were increased in the oil extracted from trees treated with calcium, especially with 200 ppm CaO NPs. An increase in total phenolic content was found in fruits treated with 200 ppm CaO NPs, while the foliar spray of the control decreased its value. The application of Nano Calcium oxide 300 ppm improved the total phenolic content of the two olive cultivars by almost 25% compared to the control treatment. CaO NPs in the concentration of 200 ppm increased the antioxidant capacity of oil by 22% and 9% in Koroneiki and Manzanillo olive oils respectively.

Table 5: Effect of nano-calcium oxide (CaO) foliar applications on the quality of olive oil. Data represent the two-year average (2022 and 2023)

CaO NPs	Free fatty acid (%)	Peroxide value (meq O ₂ / kg)	Chlorophyll (mg/kg)	Carotenoid (mg/kg)	Total phenolic content (mg/kg oil)	Antioxidant capacity (%)
Manzanillo						
Control	0.34 a	10.61 a	1.37 bc	1.11 b	46.83 b	31.18 b
100 ppm	0.36 a	9.48 ab	1.27 c	1.12 b	46.58 b	31.46 b
200 ppm	0.32 a	8.22 b	1.57 b	1.14 a	48.26 b	34.11 a
300 ppm	0.21 b	8.41 b	1.83 ab	1.31 a	58.33 a	33.94 a
Koroneiki						
Control	0.41 a	9.72 a	1.63 b	1.21 b	48.17 b	32.63c
100 ppm	0.24 b	8.37 b	1.83 ab	1.15 ab	51.32 ab	36.36 b
200 ppm	0.19 c	9.85 a	1.59 b	1.41 a	57.94 a	39.85 a
300 ppm	0.19 c	8.74 b	2.11 a	1.38 a	60.34 a	39.44 a

4. Discussion

Leaf mineral analysis is seen to be a crucial technique for future fertilization recommendations because it was utilized to assess the tree's nutritional state in terms of balance, excess, or deficiency (Fernandez-Escobar *et al.* 2011). There have been reports of increased calcium concentration in the leaves of pomegranates (Hassani *et al.* 2016) and apples (Amiri *et al.* 2008). In the current investigation, leaf mineral concentrations were higher after treatment with nano-CaO as opposed to nano-CaO treatments. The results of the experiment also demonstrated that the CaO nanoparticles promoted the formation of root hairs, which are essential for the uptake of nutrients like phosphorus that are immobile. The seedling growth rate was calculated every 15 days for a total of 30 days. The results were calculated and shown for shoot growth (Figure 9) and root growth (Figure 10). The data show that CaO nanoparticles accelerated the growth rate of seedlings. The length of the shoot and roots increased as a result of the CaO during the seedling's growth. Plants and animals absorb calcium carbonate from water to build up their skeletons and shells; this calcium carbonate is typically found in the dissolved form as calcium hydrogen carbonate Ca (HCO₃)₂. Increasing by using soluble calcium. Fertilizer application enhanced fruit yield, and there was a noticeable reaction in nano-calcium oxide, which may have something to do with calcium's effect on the uptake of nutrients by trees. Mango fruit yield has also been shown to benefit from the foliar application of calcium oxide (Sarker and Rahim, 2013). Even at low crop loads, fruit oil content did not increase in control trees, indicating that nano-fertilizers may be able to time the release of nutrient resources to coincide with crop needs. Given that nano-fertilizers have the ability to increase the reactivity of related elements more than bulk fertilizers (Naderi and Danesh-Shahraki, 2013), we postulated that applying foliar fertilizer soon after table olive fruit harvest could cause the uptake of nutrients to shift to the fruits in order to meet the metabolic needs of the cells for oil synthesis. It has also been previously observed that foliar application of calcium enhances the oil content of olive fruits (Elbadawy *et al.*, 2016).

Among calcium treatments, free fatty acid content a quality criterion for assessing olive oil exhibited a declining trend. A maximum of 0.8% is permitted by the International Oil Council (IOC) standard for this index in extra virgin olive oil. The findings demonstrated that calcium treatments decreased the peroxide value relative to the control, irrespective of their concentrations and/or formula. For this indicator, < 20 meq O₂ kg⁻¹ is the IOC norm (Mailer and Beckingham, 2006). Peroxide values above 10 often indicate less stable oil (Mailer and Beckingham, 2006). The treatment with nano-200 had the lowest values of K270 and K232. Low concentrations of K232 and K270 have been discovered to be directly correlated with oil quality (Zegane *et al.*, 2015). The polyunsaturated fatty acid-related symptom known as the K232 index primarily relates to the early oxidation of oil.

The polyunsaturated fatty acid-related symptom known as the K232 index primarily relates to the early oxidation of oil. According to Boskou *et al.* (1996), the K270 is a sign of carboxylic compounds in the oil and is connected to the secondary oxidation products. All of the treated trees produced oil with exceptional quality qualities and characteristics that were agreed upon for extra virgin oil. Jordaó *et al.*

(1990) discovered relationships between the quality of olive oil and the quantity of mineral components. Thus, in addition to its numerous functions in plant physiological metabolism, calcium fertilizer's impact on the mineral content of leaves and, more likely, fruits led to an enhancement in the chemical characteristics of oil. These results did not coincide with those published by Bouhafa *et al.*, (2014) showing that foliar application of calcium did not increase the olive oil quality. According to Dag *et al.* (2018), there is a detrimental relationship between too much calcium and olive oil acidity. Such variation in the outcomes suggests that the calcium used, how long it is applied, and the calcium level of the plant itself may all affect the quality of the oil. To date, no research has examined the use of nano-chelated calcium to enhance the qualitative characteristics of olive oil.

Consumer impression of the oil's sensory qualities includes carotenoid and chlorophyll. In the current study, the use of calcium fertilizers resulted in an increase in these pigments. As a structural element of chlorophyll, calcium is essential for the creation of fruit pigments. Our findings concurred with those of Tekaya *et al.* (2013), who observed that urea foliar spraying improved the colors in olive oil.

Biological systems are shielded from oxidative stress by the TPC, an important measure of antioxidant capacity. Additionally, it establishes the flavor, smell, and bitterness of olive oil (Bendini *et al.*, 2007). The plant's nutritional state plays a unique function in the production of TPC. The addition of calcium to the oil in the form of urea not only did not raise TPC levels, but it actually fell to the control level. However, nano-N (and specifically nano-N2) enhanced this characteristic. According to Tarafdar *et al.* (2015), the foliar spray produced phenolic synthesis most likely via known processes in nano fertilizers, such as targeted delivery, chemical characteristics, and precisely dispersed nutrients in response to crop needs and environmental stressors. In addition to the impact of calcium on the biochemical parameters of olive oil, it is important to consider the effects of chelating reagents and other accompanying compounds in nano-chelate fertilizers (Nazaran, 2012). The ability of olive oil to scavenge free radicals was used to measure its antioxidant capacity (Covas *et al.*, 2006). According to reports, the application of nanomaterials has led to an elevated accumulation of antioxidant chemicals in plant tissues (Corral-Diaz *et al.*, 2014).

The nutritional value and quality of olive oil are significantly influenced by the composition of its fatty acids. The levels of oleic and palmitic acid showed a reversal trend in response to nano-N2. According to Beltrán *et al.* (2005), the main unfavorable saturated fatty acid in olive oil is palmitic acid, while oleic acid is the most significant monounsaturated fatty acid.

It was discovered that applying 200 g L⁻¹ of nano calcium oxide topically could boost the production and oil content of olive fruits. The application of nano nitrogen enhanced antioxidant capacity and TPC, two characteristics of oil quality. The constant release of calcium from Nano fertilizers, which matched the release timing with the plant's long-term mineral uptake pattern, had an impact on these parameters, as well as by enhancing the ability of nutrients to be transported into tissues. However, since chelating agents are a necessary component of Nano fertilizers, it is important to consider their possible impacts. Based on the encouraging outcomes, orchard management strategies targeted at generating premium oils should incorporate calcium application, particularly in the form of nano calcium.

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