Effect of ZnO Nanoparticles on the Content of Sulforaphane in *Broccoli* Plant

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Abstract

The experiment was carried out during the agricultural season 2020-2021 and included two laboratory experiments. The first was carried out in the laboratories of the Department of Chemistry Sciences / College of Education for Pure Sciences / the University of Diyala, It included the preparation of nano-zinc oxide by a deposition method, and the second field was carried out in Baquba nursery / Diyala Agriculture Directorate. The addition of zinc oxide included nanoparticles at concentrations of 75 and 150 mg. L^{-1} to the nutrient solution of broccoli plants, in order to study the effect of adding different concentrations of zinc oxide nanoparticles to the sulforaphane content of broccoli by using the soilless cultivation technique. The experiment was carried out using a completely randomized design (CRD) and included a study of 4 treatments, with nine repetitions for each treatment. The averages were compared at the probability level of 0.05, the results showed that the treated plants ZnO75 mg.l^{-1} significantly by recording the highest mean of 140.µg/g fresh weight, While the average of this trait decreased in plants treated with Cooper's full salt solution and ZnO150 mg.l^{-1}, which recorded the lowest value for this trait, which were 39.25 and 41.26 µg/gm fresh weight, respectively. Compared with the control treatment Cooper's solution without zinc recorded an average of 59.59 µg/gm of fresh weight.

Key Words: Broccoli Plant, Broccoli, Nano Zinc Oxide, Sulforaphane, Hydroponics.

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Introduction

The broccoli plant is Brassica oleracea var. Italicais an important vegetable crop belonging to the cruciferous family Brassicaceae, It is one of the annual herbaceous plants that is characterized by having a wedge-shaped root and large leaves that bear on a short stem that reaches a height of 60 cm. Morphologically, it resembles cauliflower and bears at the end of the stem a group of flower buds that gather to be a large green head As well as the formation of a number of lateral heads [4 and 15]. Its cultivation is successful in soils with a pH of 5.5-6.8 [10]. Broccoli plants are divided into two groups, one group produces large white discs like cauliflower called winter cauliflower and the second consists of a number of small green or purple discs called calabrese, The broccoli plant is grown for its flowering inflorescences, which are eaten in the flower bud stage with their thick, soft pods [8]. Broccoli is a plant that is little spread in Iraq, as it ranks 31st in terms of production. Its original home is the Mediterranean Basin[6]. It was planted for the first time when the Romans ruled Italy, as well as in England in 1720 AD, and then its cultivation moved to America in 1806 AD, As for the commercial aspect, it was planted in America for the first time in 1923 AD in order to commercialize the broccoli plant in America [16].

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The broccoli plant is one of the plants with a high nutritional value because it contains many vitamins such as vitamins (A, B1, B2, B5, B6, B17, E), minerals, and carotenoids that are later converted into vitamin A inside the human body [24]. It also contains high levels of vitamin C, folic acid, riboflavin, and niacin [27]. It is also an important source of the compound sulforaphane, which has anti-cancer properties, as it has been shown that it is possible to reduce the risk of cancer by 45% when eating broccoli more than once a week and also contributed to preventing presbyopia[26]. In addition to its nutritional importance, it is a treatment and antibiotic for many common diseases, as it works to regulate the level of sugar, increases physical strength, and contributes to protecting the body from heart diseases and diseases of the urinary and reproductive system, and regulates the problem of urination [20]. Nanotechnology is one of the modern technologies that have positive effects in many fields such as agricultural, medical, engineering, as well as in the field energy[12 and 9]. This technology also contributed to reducing the use of regular fertilizers in large quantities in the agricultural field, in addition to its ability to increase production and improve plant growth [17]. In addition to the ability of nanoparticles to improve the quality and quality of plants by increasing the efficiency of nutrient absorption and eliminating diseases and pests that affect plants [14]. Zinc is one of the important, necessary, and essential micronutrients in the metabolic activities of the plant, and it has an important role in building and growing the plant through its contribution to many different biological and physiological processes, including manufacturing, metabolism, and energy formation [13]. It regulates the activity of many enzymes involved in growth, gene expression, and protein synthesis [7]. Zinc with its nano-structured structure has been widely and widely used in both foodstuffs and cosmetics and in many biological and pharmaceutical applications, as it is an antibacterial agent that regulates and enhances both immunity and growth better than traditional zinc and indirectly reduces environmental pollution [23]. Zinc oxide is used as a source of zinc, which is an inorganic compound that has many uses [25], as it is characterized by its cheap price, availability, ease of preparation, and it can bind to many materials, as well as having the property of chemical stability [2]. Because of its nutritional and therapeutic importance, much research has been conducted on the broccoli plant in order to increase its production and improve its agricultural qualities [22]. As a result of the scarcity of completed studies on the effect of zinc oxide nanoparticles in the production of sulforaphane in broccoli plants using hydroponic technology in Iraq, this experiment was carried out with the aim of:
Studying the effect of adding nano-zinc oxide on the sulforaphane content of broccoli plants.

Materials and Working Methods

Two experiments were conducted, the first was carried out in the laboratories of the Department of Chemistry Sciences / College of Education for Pure Sciences / the University of Diyala, It included the method of preparing nano-zinc oxide, and the second field method was carried out in Baquba Nursery of the Diyala Agriculture Directorate, It included the addition of nano-zinc oxide to broccoli plants, for the period from September 2020 to March 2021 in order to study the effect of adding nano-zinc oxide on the sulforaphane content of broccoli plant. The experiment was carried out using a completely randomized design (CRD), and the results were analyzed using the ready-made statistical program SAS. Duncan's polynomial test was used to differentiate between means at a probability level of 0.05[3]. The study included 4 treatments, with nine recurrences for each treatment, as shown in Table (1).

Table 1. Shows the parameters of the Experiment

No	Structural formula	transaction
1	ZnO Pre 75 $mg.l^{-1}$	Addition of nano-zinc oxide prepared by precipitation method to Cooper's nutrient solution at a concentration of 75 $mg.L^{-1}$
2	ZnO Pre 150 $mg.l^{-1}$	Addition of nano-zinc oxide prepared by precipitation method to Cooper's nutrient solution at a concentration of 150 $mg.L^{-1}$
3	Cooper's solution without zinc	Cooper's Nutrient Solution Contains all Nutrients Except Zinc
4	Cooper's full salt solution	Cooper's Nutrient Solution Contains all Nutrients Including zinc

Preparation of Nano Oxide by Precipitation Method

It was carried out according to the method mentioned by[14], as 0.1 M of aqueous zinc acetate (Zn(CH3COO)22H2O) was prepared. Then, the solution was placed on a magnetic rotor with a hot plate at room temperature, and sodium hydroxide was added at a concentration of 0.1 M to it in the form of drops until the pH became 6.7, Then the temperature of the solution was raised to 80°C to evaporate the excess water, then the remaining solution was filtered using filter paper and the precipitate was washed twice with ethanol and three times with distilled water. Then the precipitate was dried using an electric oven for two hours at a temperature of 80°C and burned at a temperature of 450°C for two hours until it became in the form of a white powder.

Determination of Sulforaphane in Fruits (µg/gm Fresh Weight)

Sulforaphane was detected using HPLC by absorbance at 254 nm. Sulforaphane was determined by its retention time and matching UV/VS approved standard. Sample quantification was performed by measuring the integrated peak area and the content was calculated using a calibration curve by plotting the peak area against the respective standard sample concentration [11] Figure (1).

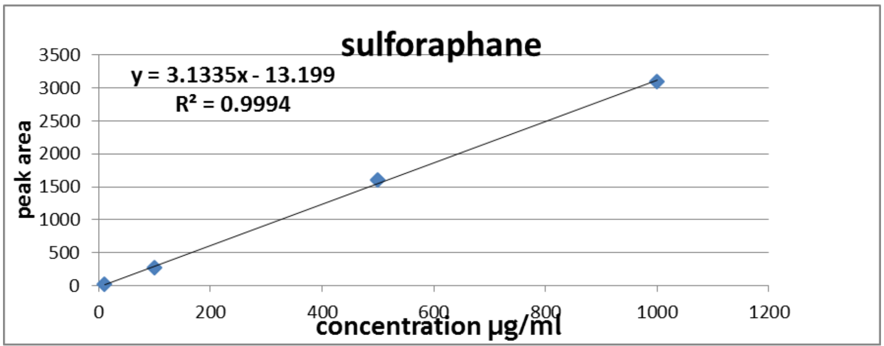


Figure 1. Standard curve of sulforaphane

The measured sample concentration was calculated based on the area under the peak curve using the following equation.

$$y = 3.1335x - 13.199$$, y = peak area, x= concentration

Discussion

Diagnosis of Nanoparticles of Zinc Oxide Prepared by X-ray

Figure (2) shows the crystal structure and phase purity of the ZnO nanoparticles prepared by the precipitation method. The results in this curve show that the higher peaks of the diffraction angles are (31.8853, 34.5393, 36.3666, 47.6483, 56.6999, 62.9592, 66.4916, 68.0469, 69.1899, 72.6770)2θ. And these peaks indicate the nature of the crystal structure of ZnO, as these peaks matched the standard values for zinc oxide nanoparticles JCPDS data card No.36-1451. The average crystal size of ZnO nanoparticles was calculated and its amount was 33.40 nm using the (Debye-Scherer equation) and these results are consistent with what was reached [19].

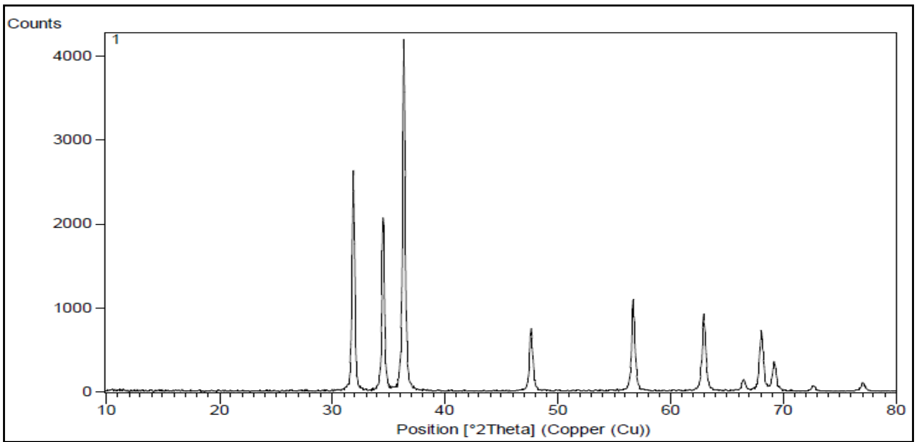


Figure 2. X-ray diffraction spectrum of nanoparticles zinc oxide

Diagnosis of Zinc Oxide Nanoparticles by Energy-dispersive X-ray Spectroscopy (EDX)

Energy-dispersive X-rays (EDX) were used to determine the components of the nano-zinc oxide as in Figure [3]. The energy-dispersive X-rays (EDX) spectrum of the product contained zinc and oxygen in a high proportion. It shows the appearance of a distinct and large peak for Zn at energy keV1 as well as the appearance of another small peak for oxygen at energy 0.5 keV, and this indicates the high purity of zinc oxide nanoparticles prepared in this way and that they do not contain impurities. The percentage of zinc was 87.1, and the percentage of oxygen was 12.9. These results are in agreement with the findings [21].

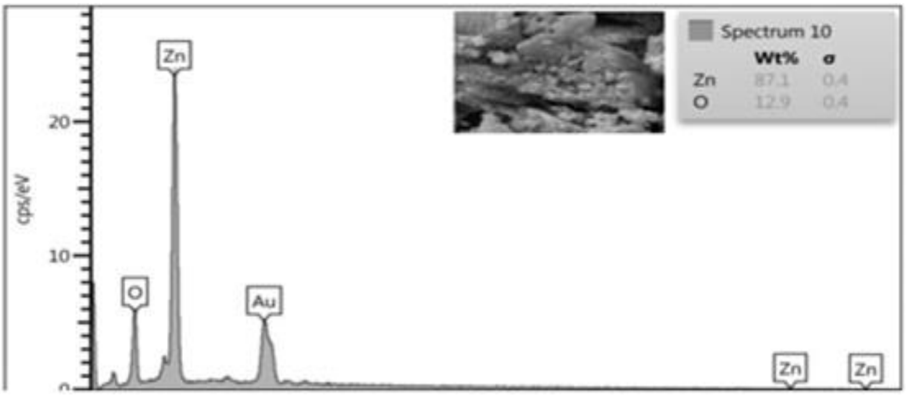


Figure 3. EDX for nano-zinc oxide

Diagnosis of Nanoparticles of Zinc Oxide by Scanning Electron Microscope (SEM)

Figure (4) shows the phenotypic and structural properties of ZnO nanoparticles prepared by deposition method using scanning electron microscopy (SEM), It is noticed that the nanoparticles were prepared in the nanometer range and the SEM images showed good separation of some nanoparticles from each other, While most of them appear agglomerated and the reason is due to the electrostatic effects in addition to the effect of the aqueous suspension, and this is consistent with a behavior similar to the agglomeration of nanoparticles in the results of previous studies, as the average diameter of these particles was about 53.52 nanometers [5].

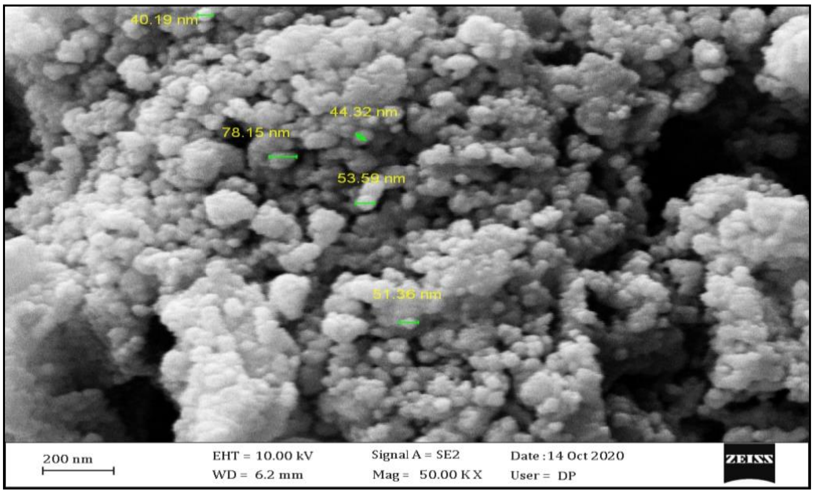


Figure 4. Scanning electron microscope images of zinc oxide nanoparticles

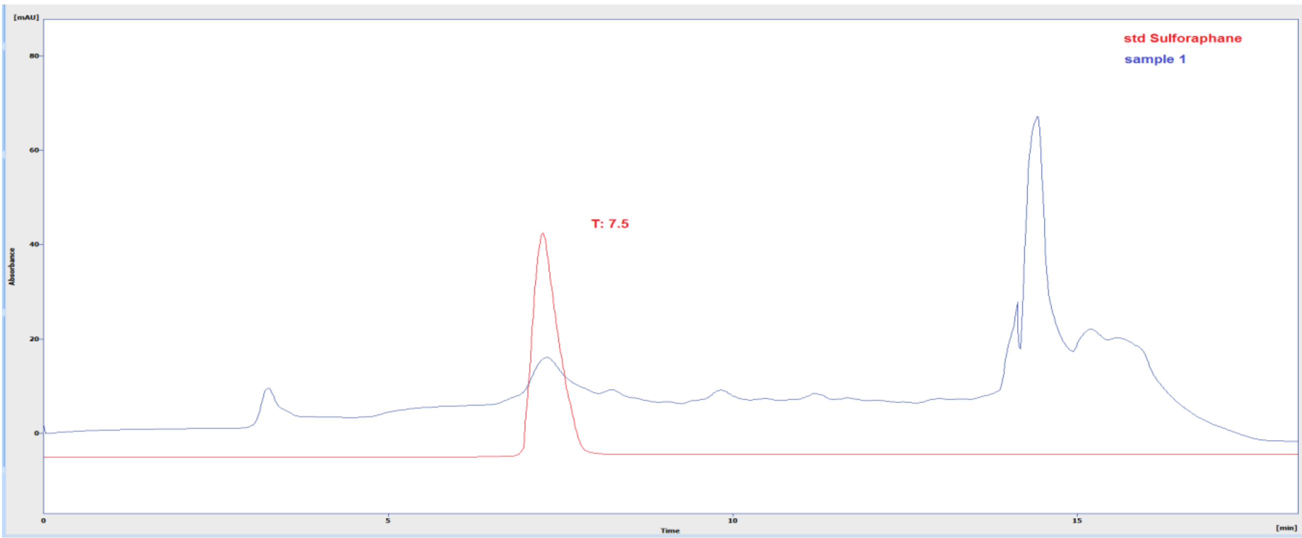
Determination of Sulforaphane in Fruits (µg/gm Fresh Weight)

The results presented in Table (3) and chromatograms in Figure (5) show that the addition of nano-zinc oxide to the nutrient solution of broccoli plants with different concentrations had a significant effect on the sulforaphane content of the fruits. The ZnO75 mg.l^{-1} treated plants achieved the highest average of 140.µg/gm fresh weight, while the mean of this trait decreased in plants treated with Copper full salt solution, which recorded the lowest value for this trait, which amounted to 39.25 µg/gm fresh weight. Compared with the control treatment Cooper's solution without zinc recorded an average of 59.59 µg/gm of fresh weight.

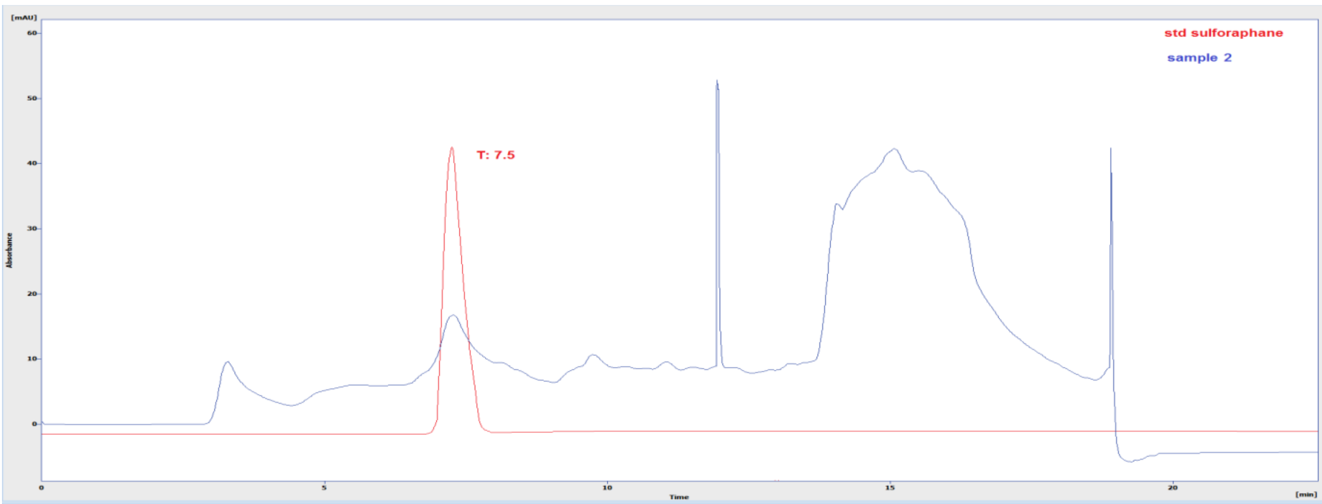
Table 3. Shows the effect of adding different concentrations of nano zinc oxide and sulfide on the sulforaphane content of broccoli plants Brassica oleraceae var. Italica growing on farms without soil

sample	Peak area	µg/ml 1	total yeald2	µg/gram fresh plant weight 3
ZnO 75 mg.l^{-1}	447.32	151.21	302.42	60.48
ZnO 150 mg.l^{-1}	297.21	103.16	206.32	41.26
Cooper's solution without zinc	440.31	148.97	297.94	59.59
Cooper's full salt solution	281.47	98.12	196.25	39.25

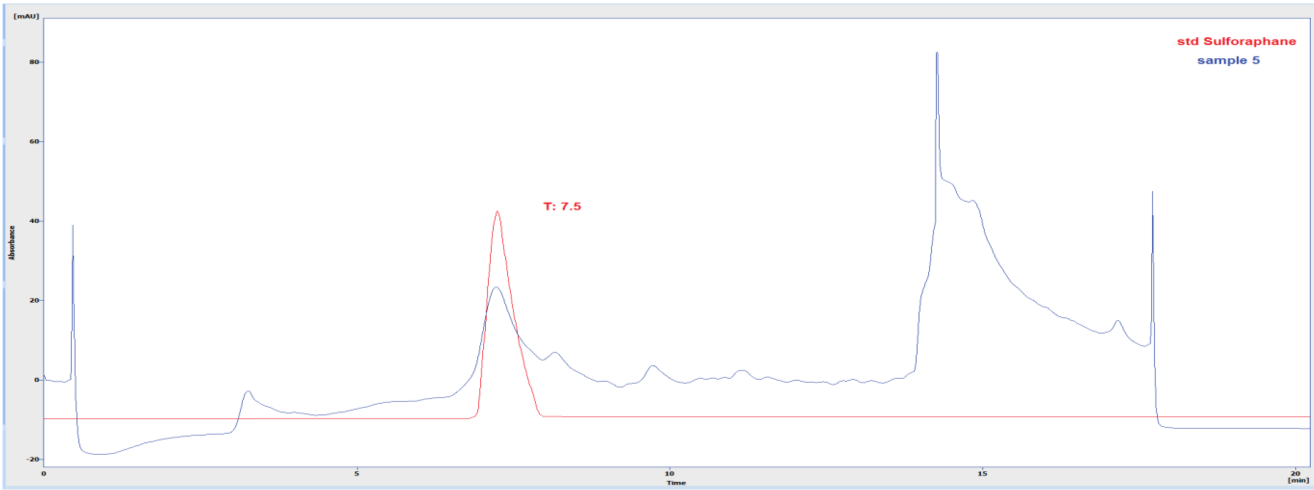
Broccoli is one of the plants famous for containing many antioxidants such as vitamin C and sulforaphane, so increasing the consumption of broccoli can reduce the risk of skin, breast, and prostate cancer, Recent studies have shown that glucosinolates4-Methylsulfinylbutyl glucosinolate is another important component of broccoli and can produce sulforaphane when hydrolyzed by myrosinase. The reason for the superiority of nano-ZnO in this characteristic to broccoli plants, as shown in Scheme A, may be due to the fact that nano-fertilizers provide larger areas for various metabolic activities in the plant, which in turn increases the process of photosynthesis (18). This, in turn, increases the number of processed carbohydrates that are transmitted to the leaves and the pink disc, and thus increases the content of sulforaphane resulting from those carbohydrates.



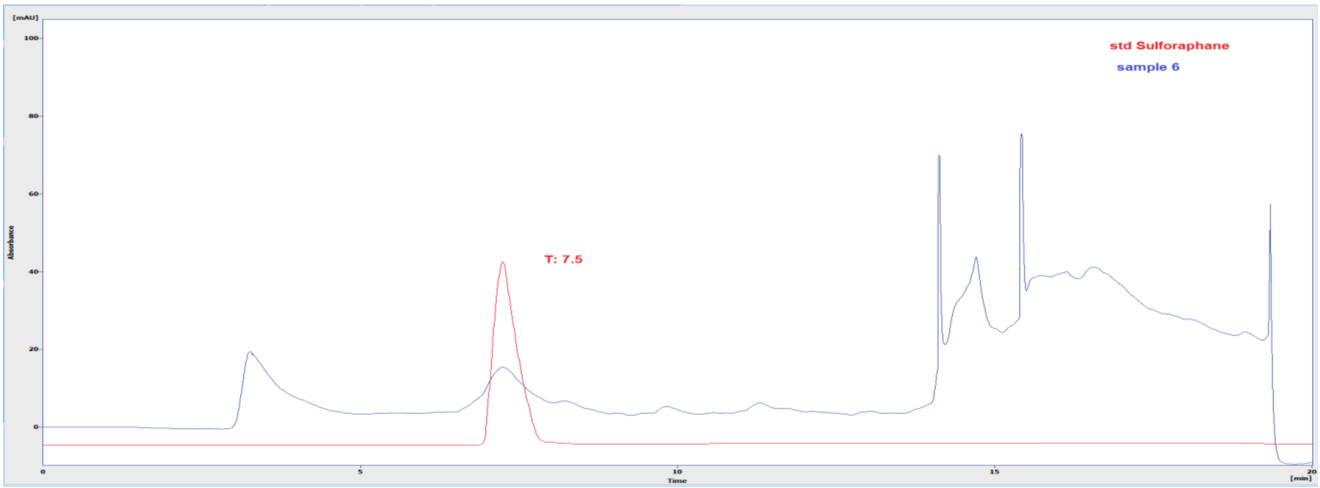
A. Adding a concentration of ZnO 75 mg.l^{-1}



B. Adding ZnO concentration 150 mg.l^{-1}



C. Cooper's solution without zinc



D. Cooper's full salt solution

Figure 5. Chromatograms of sulforaphane content in fruits when adding nano-zinc oxide with different concentrations to Brassica oleraceae var. Italica growing in hydroponics

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