



Effect of Spraying with different Concentrations of Methyl Jasmonate on the Sulforaphane Content of Broccoli Plant

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Abstract

The experiment was carried out during the 2020-2021 agricultural season in the greenhouse of the Baquba nursery/ Diyala Agriculture Directorate, with the aim of studying the effect of spraying with different concentrations of Methyl Jasmonate on the sulforaphane content of the broccoli plant using the soilless cultivation technique. The experiment was implemented using a completely randomized design (CRD) and the study included 4 treatments with nine repetitions for each treatment. The averages were compared at a probability level of 0.05. The study included foliar spraying of broccoli at concentrations of 25,50,75 $\mu\text{mol. l}^{-1}$ of Methyl Jasmonate As well as a control treatment, the results showed the surprise treatment of the MeJA compound by focusing 25 $\mu\text{mol. l}^{-1}$, While the average of this trait decreased in the treated plants MeJ50 $\mu\text{mol.l}^{-1}$, which recorded the lowest value for this trait, which was 59.57 $\mu\text{g/gm}$. Compared to the control MeJA0 $\mu\text{mol.l}^{-1}$, which recorded a mean of 125.31 $\mu\text{g/g}$.

Key Words: Broccoli Plant, Methyl Jasmonate, Sulforaphane, Foliar Spray.

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Introduction

The broccoli plant is *Brassica oleracea* var. *Italica* is one of the most important vegetable crops belonging to the cruciferous family Brassicaceae, and it has many English names, including Sporouting Cauliflower, Asparagus Italian, Broccoli, and it is also called Calabrese in the UK⁽⁸⁾. The broccoli plant is grown for its flowering inflorescences, which are eaten in the flower bud stage with their thick, soft pods⁽⁴⁾. The original home of the broccoli plant is the Mediterranean basin⁽²⁾. It was planted for the first time when the Romans ruled Italy, as well as in England in 1720 AD, and then moved to America in 1806 AD, but from a commercial point of view, it was planted in

America for the first time in 1923 AD in order to commercialize the broccoli plant in America⁽⁹⁾. 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 transformed into vitamin A inside the human body⁽¹⁴⁾. It also contains high levels of vitamin C, folic acid, riboflavin, and niacin⁽¹⁷⁾. 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 several times a week and also contributed to preventing presbyopia⁽¹⁶⁾.

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In addition to its therapeutic and nutritional value that is not found in another plant, it is a treatment and antibiotic for many common diseases, as it helps to regulate the level of sugar, increases physical strength, helps protect the body from diseases of the heart, urinary and reproductive tracts, and regulates the problem of urination ⁽¹¹⁾. Methyl Jasmonate (MeJA) is one of the hormones used in preparing plants and inducing them to build defense compounds and express disease-related genes involved in acquired and endogenous resistance systems therefore it is used against pathogens, both water and salt stress, low temperature and heavy metals and elements stress other toxic, It also improves growth, induces the accumulation of active compounds, and affects the level of endogenous hormones and other physiological and biochemical properties in stressed plants⁽¹⁵⁾. 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 ⁽¹³⁾. As a result of the scarcity of completed studies on the effect of Methyl Jasmonate in the production of sulforaphane in broccoli plants using hydroponic

technology in Iraq, this experiment was carried out with the aim of Study of the effect of spraying with Methyl Jasmonate on the sulforaphane content of broccoli plants.

Materials and Working Methods

A field experiment was conducted in Baquba Nursery of Diyala Agriculture Directorate for the period from September 2020 to March 2021 in order to study the effect of spraying with Methyl Jasmonate on the sulforaphane content of broccoli plant. This experiment included studying the effect of spraying with Methyl Jasmonate at concentrations (25,50,75) micromol.l⁻¹, where the experiment's treatments were sprayed until the stage of superficial gonorrhoea and by two sprays the first one month after transferring the plants and the second spray three weeks after the first spray (flowering stage), The experiment was carried out using the Completely Randomized Design (CRD) and the study included 4 treatments, with nine repetitions for each treatment, as shown in the table below.

A table showing the parameters of the experiment

T	Treatment Structural Formula	transaction
1	MeJA 0 $\mu\text{mol.l}^{-1}$	Spraying with Methyl Jasmonate at a concentration of 0 micromol.l ⁻¹
2	MeJA 25 $\mu\text{mol.l}^{-1}$	Spraying with Methyl Jasmonate at a concentration of 25 $\mu\text{mol.l}^{-1}$
3	MeJA 50 $\mu\text{mol.l}^{-1}$	Spraying with Methyl Jasmonate at a concentration of 50 $\mu\text{mol.l}^{-1}$
4	MeJA 75 $\mu\text{mol.l}^{-1}$	Spraying with Methyl Jasmonate at a concentration of 75 $\mu\text{mol.l}^{-1}$

Determination of Sulforaphane in Fruits ($\mu\text{g/gm}$ Fresh Weight)

5 g of fresh broccoli fruits were crushed for 5 minutes and left for autolysis at room temperature for 30 minutes, after autolysis, the plant tissue was extracted twice with 50 ml of methylene chloride mixed with 2.5 g of anhydrous sodium sulfate. The methylene chloride fraction was evaporated at 30 °C using a rotary evaporator. The remainder of the extract was dissolved in acetonitrile and then filtered through 0.22 mm micro filters before injection into the HPLC device. Separation was carried out using a C18 (250X4.6) separating column with 5 μm particles in size.

Separation Process

The solvent system consists of 20% acetonitrile in water; This solution was then linearly changed over 10 minutes to 60% acetonitrile and maintained at 100% acetonitrile for 2 minutes to disinfect the column. The column temperature was set at 30°C. The flow rate was 1 mL/min, and 20 μL portions per column. Sulforaphane was detected by absorbance at 254 nm. Sulphoraphane was determined by its holding time and UV/VS Spectrophotometric match. 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⁽⁶⁾.



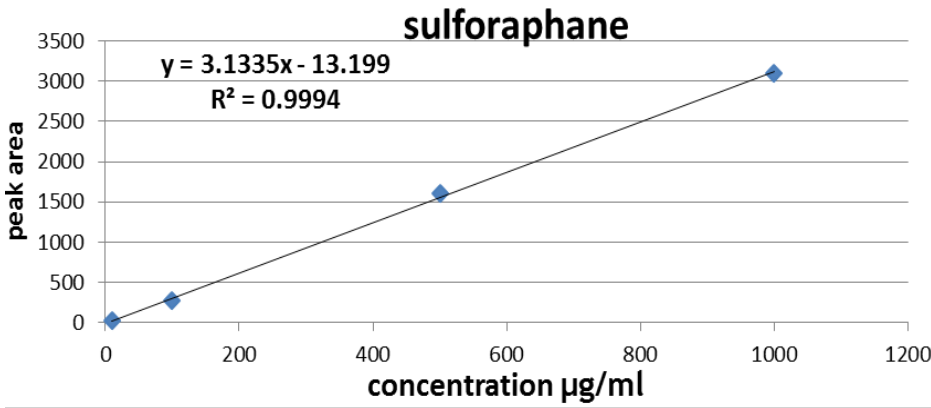


Figure. Showing the standard curve of sulforaphane

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

Results

The results presented in the table and charts below show that foliar spraying of broccoli plants with different concentrations of Methyl Jasmonate had a significant effect on the sulforaphane content of the fruits. The plants of the treatment MeJA25 µmol.l⁻¹ achieved the highest average of 140.12 µg/gm (curve C), while the mean of this trait decreased in the plants of the MeJA50 µmol.l⁻¹ treatment, which recorded the lowest value for this trait of 59.57 µg/gm (curve B). Compared to the control

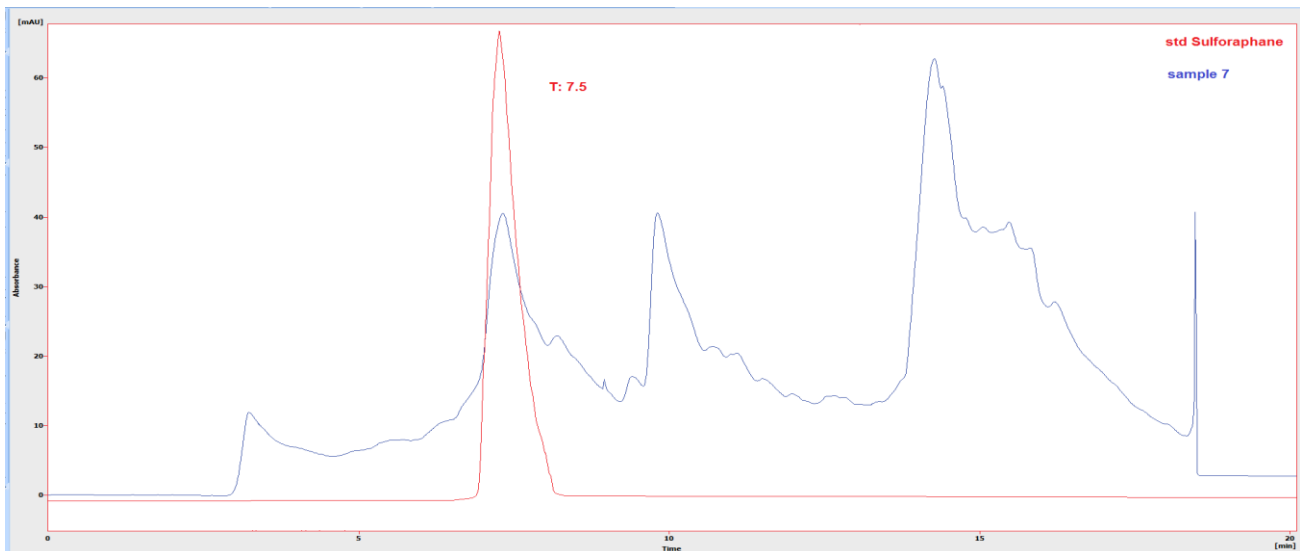
treatment MeJA0 mg.l⁻¹, which recorded a mean of 125.31 µg/g (curve D).

Table showing the effect of foliar spraying with different concentrations of the same on the content of sulforaphane in the fruits of broccoli plants *Brassica oleraceae* var. Italica growing on farms without soil

sample	Peak area	µg/ml 1	total yeald 2	µg/gram fresh plant weight 3
MeJA 0 µmol.l ⁻¹	953.63	313.28	626.56	125.31
MeJA 25 µmol.l ⁻¹	1069.26	350.29	700.58	140.12
MeJA 50 µmol.l ⁻¹	440.22	148.94	297.87	59.57
MeJA 75 µmol.l ⁻¹	696.94	231.11	462.23	92.45

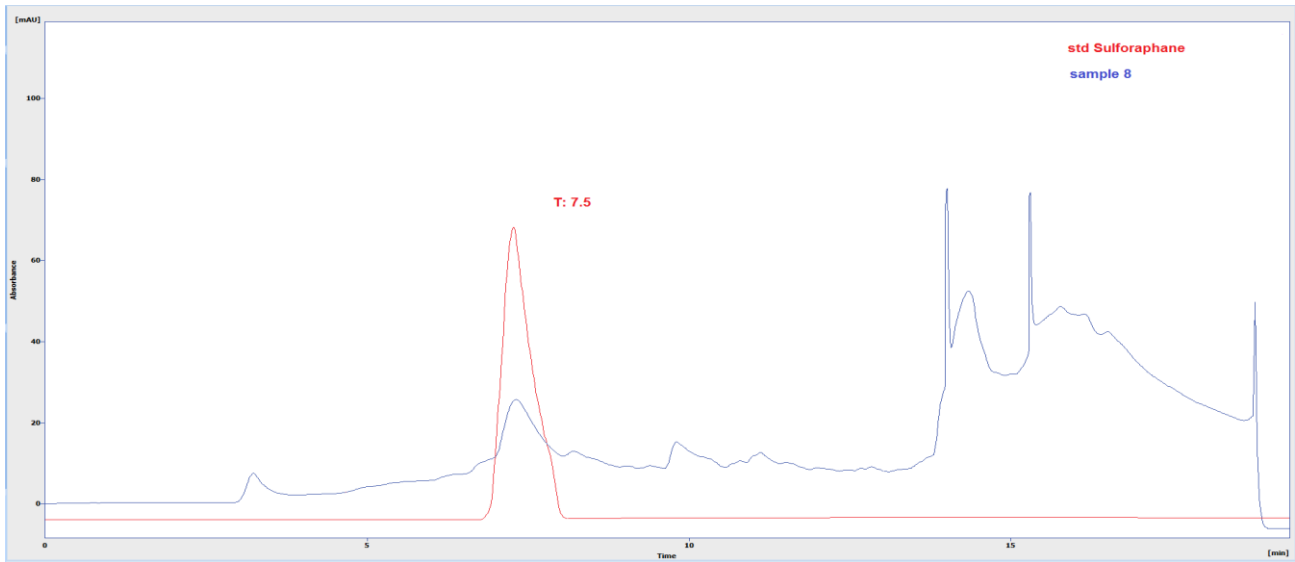
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Chromatograms of sulforaphane content in fruits when foliar spraying with different concentrations of Methyl Jasmonate for broccoli plant *Brassica oleraceae* var. Italica growing in hydroponics.

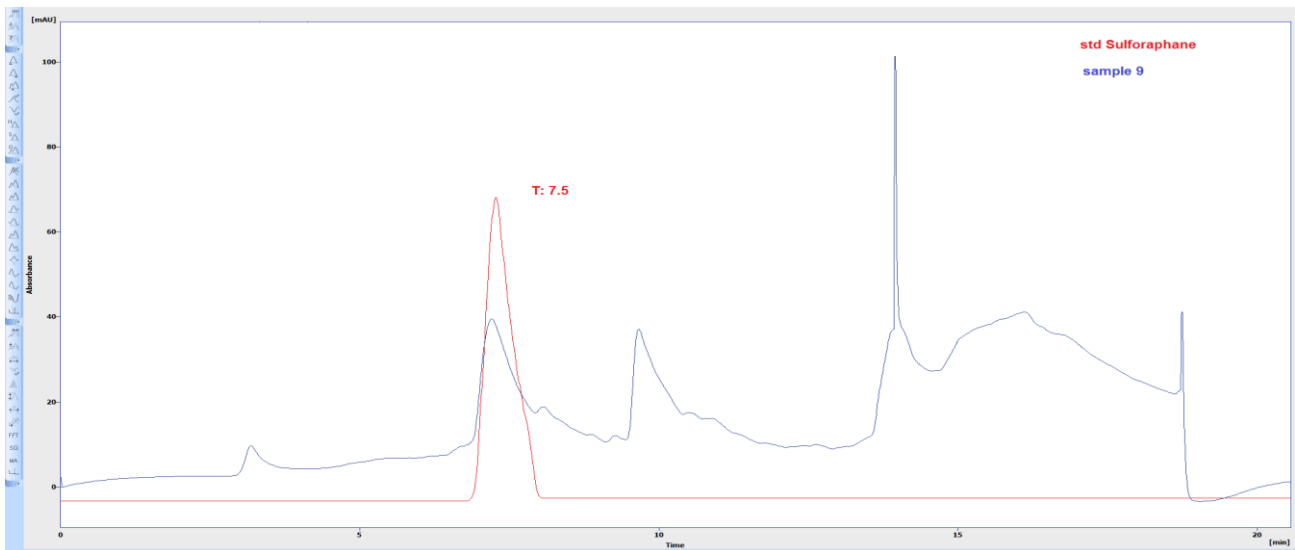


A: Spraying at a Concentration of MeJA75 µmol.l⁻¹

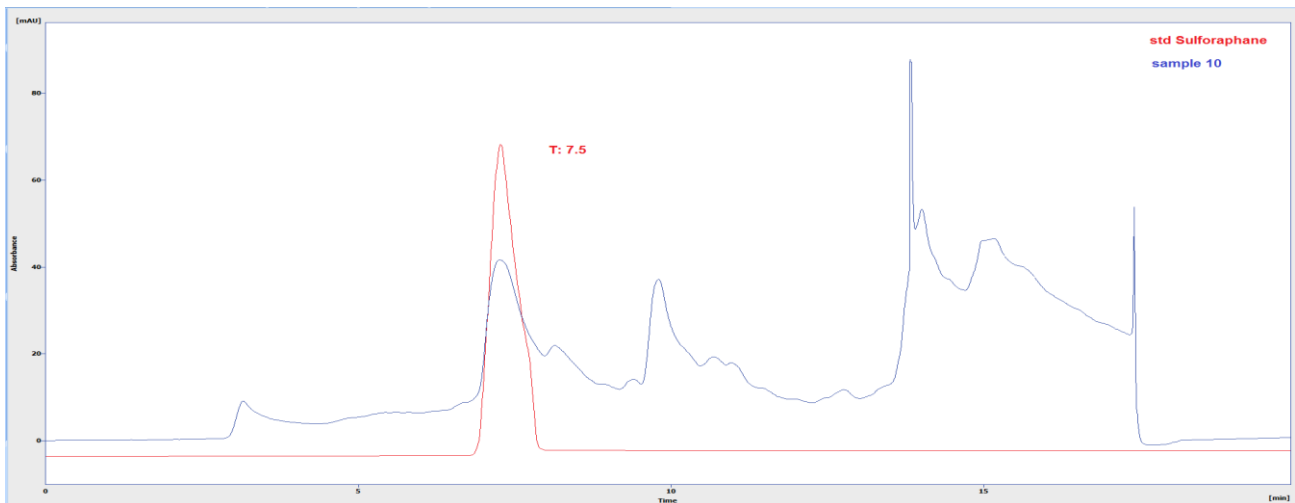




B: Spraying at a Concentration of MeJA50 $\mu\text{mol.l}^{-1}$



C: Spraying at a Concentration of MeJA25 $\mu\text{mol.l}^{-1}$



D: Spraying at a Concentration of MeJA0 $\mu\text{mol.l}^{-1}$



Discussion

One of the useful biological techniques is the addition of exogenous stimuli to excite plant cells to produce secondary metabolites of medical and therapeutic importance⁽³⁾. The reason for the increase in the content of sulforaphane in fruits when spraying with low concentrations of Methyl Jasmonate may be attributed to its role in activating genes that synthesize ribosomal DNA, mRNA, which encodes enzymes responsible for the production of secondary compounds⁽⁶⁾. The example of Methyl Jasmonate works as an abiotic catalyst that stimulates the flow of many reactions of secondary metabolic compounds in the form of a defensive response in plants, by activating catalytic reactions with specific enzymes that enter the process of biosynthesis of secondary compounds^(10,7,12). The significant decrease in sulforaphane content with increasing concentration of Methyl Jasmonate may also be attributed to the latter's conversion of sulforaphane biosynthesis pathways to other secondary compounds such as phenolic compounds⁽¹⁾.

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