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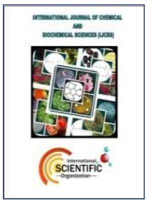

The Effects of Boron Combined with Tryptophan or Reduced Graphene Oxide on Growth, Productivity and Fruit Chemical Composition of *Foeniculum vulgare* Mill

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Abstract

A two-season field experiment was conducted to investigate the effect of boron foliar application at 0, 100, and 200 ppm enhanced with reduced graphene oxide (rGO) at 3 and 6 g/L or tryptophan at 100 and 200 ppm on plant growth, fruit yield, and essential oil production. Of the 15 total treatments, the foliar application of boron (at 100 ppm) combined with rGO (at 6 g/L) or tryptophan (at 200 ppm) resulted in the tallest, most branched plants and the highest fresh and dry weights which was reflected in the number of umbels and fruit yield. The combination of boron with tryptophan was found to result in a higher oil yield than that achieved with rGO. The PCA analysis showed a strong correlation between fruit yield and both the number of umbels per plant and the fresh and dry weight. However, a low correlation was observed between essential oil yield and growth parameters. In conclusion, the combined application of boron at 100 ppm with either rGO at 6 g/L or tryptophan at 200 ppm has been found to elicit a synergistic and positive response, by improving the growth and productivity of fennel.

Keywords: *Foeniculum vulgare*; amino acids; nanomaterials; micronutrients; growth parameters; essential oil.

Full length article

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1. Introduction

The aromatic annual herb fennel (*Foeniculum vulgare* Mill.) is a member of the *Apiaceae* family [1]. It is native to the Mediterranean and Asia Minor, but it has been introduced to a wide range of tropics and subtropics. Depending on its genotype or botanical type, fruits of the plant contain approximately 42.3% carbohydrates, 18.5% fiber, 13.4% minerals, 10% protein, and 0.7-6 % essential oils [2]. There are a number of different morphological and chemical characteristics of fennel. Based on morphological data alone, some genotypes of fennel listed in the European Pharmacopeia cannot be distinguished. However, several studies have recognized a number of distinct chemotypes according to the relative concentrations of key compounds in essential oils, including estragole and (E)-anethole [3]. Some of the applications of fennel essential oil include its use as a flavoring agent and as a carminative agent in laxative preparations [4]. Mahfouz and Sharaf-Eldin [5] note that fennel has strong anti-inflammatory and antispasmodic effects. It also has various uses in traditional medicine as a sedative, diuretic, carminative, stimulant, expectorant, galactagogic, antispasmodic, and emmenagogic [6]. Furthermore, fennel fruits are used as a popular seasoning and culinary spice in the food industry [4]. Fennel plants need both macro and micronutrients for the best growth and development. Among the micronutrients that are important in the regulation of metabolic processes is boron. Boron is essential for leaf photosynthesis, cell division, and nitrate metabolism [7]. For example, turmeric exhibited a significant decrease in stomatal conductance when subjected to boron deficiency [8]. Boron showed a significant influence on the fruit set of hazelnut [9], and it was associated with anther's pollen-producing capacity, pollen viability, pollen tube germination, and pollen tube growth of sesame plant [10]. The presence of boron in cell membranes may affect the physical properties of membrane proteins in addition to its critical role in ion uptake [11 & 12]. Shireen *et al*. [7] reported that boron enhances crop growth and yield in many plants. In turn, [13] found that foliar application of boron on soybean increased seed yield, number of pods per branch, and number of seeds per pod. In recent years, there has been significant development in the scientific discipline of 'plant nano-bionics'. This field utilizes natural or synthetic nanomaterials to influence plant growth and development, physiological processes, and the control of nutrient and fertilizer release [14]. Among the various types of carbon-based nanomaterials, graphene, graphene oxide nanosheets (GNS), and reduced graphene oxide (rGO) are included [15]. Several reports have discussed the effects of GNS on plants [16,17], and their safety in the agricultural sector for improving the growth and yield of important horticultural crops, such as *Capsicum annuum* and *Solanum melongena* [18]. The application of graphene quantum dots has been shown to enhance the growth of coriander and garlic [19]. Another promising application for GNS and rGO in the agricultural sector is their use as a carrier material for nutrients, facilitating their slow release for the plant in a controlled and gradual manner [20]. This results in a significant reduction in the loss of nutrients and an improvement in soil properties [21]. Consequently, the total chlorophyll content, comprising chlorophyll a and b is increased, which promotes plant vegetative growth [22]. Additionally, rGO can be employed to mitigate lead phytotoxicity [23]. In the context of sustainable crop protection, reduced graphene oxide can be employed as an eco-friendly nanopesticide and highly effective biocide, offering a viable alternative to conventional chemicals that may exert phytotoxic effects [20,24]. Carbon nanotubes (CNTs) are another type of carbon-based nanomaterial that has been used in agriculture to enhance plant growth. Studies have shown that CNTs positively affect onion and cucumber root growth [25], the total fresh biomass of tobacco [26], root length in wheat seedlings [27], and the germination and growth of soybean, maize, and barley [28]. Nevertheless, some negative effects have been reported for CNTs in certain plant species, such as acute cytotoxicity and genetic modification [29]. In mustard plants, this effect has been demonstrated to be dose-dependent, resulting in high cytotoxicity and reduced germination and biomass at high concentrations [30]. Therefore, it is necessary to assess the potential risks of using CNTs for various applications on plant growth.

Intensive crop production aimed at improving quality and yield, as well as regulations introduced in several countries worldwide, are prompting the use of natural products, protein hydrolysates having free amino acids. The amino acids are defined as organic nitrogen compounds that combine to form proteins. They regulate plant growth and take part in the synthesis of important chemicals necessary for plant growth. In this context, certain amino acids that are closely linked to various metabolic pools in plants have been utilized to enhance plant growth [31]. Tryptophan is a precursor of auxins, which manage the elongation rate of stems and roots and the opening of leaf buds. It is also involved in the biosynthesis of phytoalexin camalexin, phenylpropanoids and other related natural products [32]. This finding confirms the direct role of tryptophan in plant growth by its influence on the synthesis of auxins [33]. It has been found that plants respond to exogenously applied tryptophan because of insufficient endogenous auxin biosynthesis. The foliar application of tryptophan has also been shown to enhance the vegetative growth and productivity of several plant species including *F. vulgare* [34,35] and *Rosmarinus officinalis* [36].

The effect of combining boron foliar treatment with reduced graphene oxide or amino acids on fennel growth and production is currently poorly understood due to the limited availability of relevant information. Therefore, the objective of this study was to improve the performance of field-grown fennel by combining boron, rGO, and tryptophan at different concentrations and to investigate the effects of their application on fennel morphology, plant growth, fruit production, and essential oil yield. This will facilitate the identification of the optimal combination of these nutrients and their optimal concentrations, which will be of significant benefit to commercial growers.

2. Material and Methods

2.1. Plant material.

Seeds of a local variety of fennel (*Foeniculum vulgare* Mill.), traditionally cultivated in Egypt, were obtained from Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agriculture Research Centre, Giza, Egypt. This variety is extensively grown in Egypt due to its good growth and high productivity, as well as its suitability for the soil conditions and climate of the central and southern regions of the country.

2.2. Treatments and experimental design

A field trial was conducted in two consecutive seasons 2020/2021 and 2021/2022 at the farm of the Faculty of Agriculture, Al-Azhar University, Assiut Branch, Egypt. The physical and chemical characteristics of the used soil revealed that the soil texture was clay (22.3 sand, 26.2 silt and 51.5% clay), with a pH of 8.71 (1:2.5 soil suspension in distilled water), electrical conductivity (EC) of 1.03 dS/m (1:5 soil solution), total $CaCO_3$ of 1.97%, organic matter of 0.97%, total N of 0.7%, total P of 0.21% and total K of 41%. Soluble ions (meq/L, soil paste) included Cl^- (3.32), HCO_3^- (4.49), SO_4^{2-} (3.05), Ca^{+2} (5.40), Mg^{+2} (0.52), Na^+ (1.30) and K^+ (3.89). The field experiment was laid out in a split-plot design in three replications.

The following factors were analyzed in the experiment:

I – boron applied at concentrations: 0, 100 200 ppm obtained by dissolving 0, 0.571, and 1.142 g/L of Orthoboric acid powder, (H3BO3; M.W. 61.83; Oxford Lab Chem, Mumbai, India) in distilled water. It was designated to the main plots in a random manner

II – reduced graphene oxide (rGO: CxHyOz, Ossila Ltd, Solpro Business Park, Windsor St., Sheffield S4 7WB, UK) of 3 and 6 g/L (applied to subplots)

III - tryptophan (L-tryptophan, C11H12N2O2, MW: 204.23, Oxford Lab Chem, Mumbai, India) of 100 and 200 ppm dissolved in distilled water (applied to subplots).

Thus, the following 15 combinations were obtained: 1) B0, 2) B0rGO3, 3) B0rGO6, 4) B0T100, 5) B0T200, 6) B100, 7) B100rGO3, 8) B100rGO6, 9) B100T100, 10) B100T200, 11) B200, 12) B200rGO3, 13) B200rGO6, 14) B200T100, 15) B200T200

Each treatment included three replications, and each experimental unit (plot) contained three rows 1.25 m wide and 1.8 m long. All the treatments had the same row spacing of 0.25 m. In the middle of November of each growing season, seeds were sown, and after 35 days, thinning was done by leaving a single plant per hill, resulting in 15 plants for each experimental unit. After 10 days of thinning, treatments of boron were applied as a foliar spray. Meanwhile, treatments of rGO and tryptophan were sprayed one day later to avoid leaching influence. The total volume applied from each treatment was 2000 mL per experimental unit (plot) and the control plants were sprayed with distilled water only. These treatments were repeated two more times every 15 days. All other field practices were followed as recommended, including irrigation, weeding and fertilization. The fertilization program included the application of superphosphate at a rate of 200 kg/feddan (0.42 ha) during soil preparation about one month before seed sowing. Nitrogen was applied in the form of $(NH_4)_2SO_4$ at a rate of 200 kg/fed in two doses; the first dose (100 kg/fed) was applied directly after thinning of seedlings 25 days after sowing, whilst the second dose (100 kg/fed) was applied 45 days after the first dose. The potassium was applied as K_2SO_4 at a rate of 50 kg/fed with the second dose of nitrogen fertilizer.

2.3. Plant growth measurements.

At the end of the growth season (at the fruit maturity stage after 160 days of sowing), data were collected on vegetative growth parameters of all 15 plants from each experimental plot. This included plant height, number of branches and fresh and dry weights. The dry weight of the stem was determined after oven-drying at a temperature of 70 °C until a constant weight was reached. Intensity of flowering (number of umbels per plant) was recorded and fruit yield (fruit weight in tons per feddan [0.42 ha]) was calculated. Fruits were harvested at the maturity stage when turning from green to brown color and were left to air-dry under shade conditions for 1 week after which the weight was averaged in grams per plant. Based on the plant spacing applied in the current experiment (15 plants in 2.25 m^2), the yield was calculated per feddan (0.42 ha, 4200 m^2) in tons.

2.4. Essential oil yield

Air-dried fruits were used to obtain essential oil through hydrodistillation using the Clevenger-type apparatus [37]. Fruit samples (100 g) representing the three replicates per treatment were ground before being subjected to distillation. The ground fruits were placed in distilled water, and then hydrodistilled for 3 h in a Clevenger-type apparatus. After collecting the essential oil, it was dried with Na_2SO_4 and stored at 4 °C until GC/MS analysis was performed. The percentage of distilled essential oils was recorded and the oil yield per plant was then calculated. This was used to calculate the oil yield per feddan (0.42 ha).

2.5. GC-MS analysis of essential oil

A GC-MS analysis was conducted on the essential oil obtained from three treatments, including the control and the two most efficient treatments, i.e., boron at 100 ppm combined with nano-carbon material at 6 g/L, and boron at 100 ppm combined with tryptophan at 200 ppm. The chemical composition of the essential oil was determined using a Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX. USA) coupled with TG-5MS capillary columns (30 m x 0.25 mm x 0.25 µm film thickness). Initially, the column oven temperature was set to 50 °C and then increased by 5 °C/min. to 150 °C and maintained for 2 min. After that, the temperature was increased by 5 °C/min. to 250°C and held for 2 min. The injector was run at 270 °C, while the MS transfer line was run at 260 °C. As the carrier gas, helium was injected at a rate of 1 mL/min. Injection of diluted samples (1:19 v/v with hexane) was automated after a 4-min. solvent delay using the autosampler ASI300 in split mode. The mass spectra were collected using 70 eV ionization voltages in full scan mode. The ion source temperature was set at 200 °C. To identify the components, the mass spectra of each component were compared with those of WILEY 09 and NIST 14. The quantitative data were expressed as the GC peak area percentage of the oil constituents calculated relative to the total peak area.

2.6. Statistical analysis

The data were statistically analyzed by analysis of variance (ANOVA) for split-plot design, in which the boron, rGO, tryptophan and their combinations (boron with rGO and boron with tryptophan) were considered. The least significant difference (LSD) test at the significance level p=0.05 was used to determine the differences among the means of treatments [38]. Statistix 8.1 software [39] was used to conduct statistical analyses. A principal component analysis (PCA) was conducted on growth parameters (plant height, number of branches, fresh and dry weights, number of umbels per plant and fruit yield) as well as essential oil percentage and yield) to identify those parameters that were closely linked to each other and to determine which treatments, specifically boron with reduced graphene oxide and tryptophan, largely influenced these parameters. The PCA analysis was performed using Analyse-it Software (v. 5.6 for Excel).

3. Results and discussion

3.1. Growth attributes

Foliar application of boron (B) alone and in combination with tryptophan or rGO at various concentrations had a significant effect on plant height, number of branches, fresh and dry weights of *F. vulgare* (Fig. 1-5). Applying boron at a concentration of 100 ppm led to a significant increase in plant height in both growing seasons compared with that recorded for the control plants (Fig. 1). In contrast, no differences were noticed when boron was added at a concentration of 200 ppm. A significant improvement in plant height in 2020/2021 and 2021/2022 was also observed in plots treated with rGO at 3 and 6 g/L. The application of tryptophan at 100 ppm resulted in a significant increase in plant height compared to the control and other treatments of tryptophan and rGO in both seasons. However, it was the application of boron at 100 ppm in combination with tryptophan at 200 ppm that resulted in the tallest plants in the experiment.

The results of the study indicated that the foliar treatment with different boron concentrations did not affect the number of fennel branches over two growing seasons (Fig. 3). Only the treatment with tryptophan alone at 200 ppm, rGO alone at 6 g/L, as well as treatment with the combination of boron 100 ppm plus tryptophan or rGO, significantly stimulated branching when compared with the control (non-treated plants). The combined application of B 100 ppm plus rGO 6 g/L significantly stimulated the number of branches in *F. vulgare*, which depending on the growing season, reached 11 and 11.80 branches per plant in 2020/2021 and 2021/2022, respectively. It is worth noting that a similar effect on fennel branching was achieved by the application of tryptophan alone at 200 ppm.

The fresh and dry weights of the control *F. vulgare* plants, which amounted to 581.6 and 166.8 g/plant in the first season and to 598.2 and 171.2 g/plant in the second season, was significantly increased to 674.2 and 190.7 g/plant in the first season and to 687.3 and 196.7 g/plant in the second season by foliar application of boron at 100 ppm (Fig. 4 & 5). There were no significant differences in fresh and dry weight between control (non-treated) plants and plants treated with boron at 200 ppm. In contrast, *F. vulgare* treated with rGO (6 g/L) or tryptophan (200 ppm) alone, produced significantly more fresh and dry weights in both growing seasons than control (non-treated) plants, as well as plants treated with a combination of rGO at 3 g/L or tryptophan at 200 ppm with 200 ppm of boron. In general, the application of 100 ppm boron in combination with 6 g/L rGO had the best effect on the fresh and dry weights of *F. vulgare*, which was 771.4 and 222.4 g/plant in the first growing season while 792.6 and 229.6 g/plant in the second season, respectively (Fig. 4,5).

In this study, the application of different concentrations of boron, reduced graphene oxide (rGO) and tryptophan as treatments, resulted in a significant improvement in the growth and yield characteristics of *F. vulgare*. This was evidenced by a significant increase in plant height, fresh and dry weights, and the number of branches. The addition of boron through fertilization can, in many instances, enhance crop productivity by mitigating the adverse effects of elevated heavy metal concentrations, thereby reducing overall yield losses. The results of our study indicate that boron has a stimulating effect on the growth of *F. vulgare*. This result is in accordance with the findings of [40], who demonstrated that the application of boron at concentrations of 40 and 80 ppm enhanced the vegetative growth of *F. vulgare*. This finding is also consistent with the results of several recent studies conducted on various members of the family *Apiaceae*, to which *F. vulgare* belongs. Abdallah and coauthors [41] found that the foliar application of boron at 150 ppm led to a significant improvement in the growth of *Coriandrum sativum* plants compared to untreated plants. The increment due to boron application reached 27 % in plant height, more than 100 % in the number of branches and 25% in dry weight. Several other crops such as olive, highbush blueberry, barley, wheat, tomato and *Arabidopsis thaliana* have also shown better growth in response to boron application [42,43,44,7]. The enhancement in plant growth due to the application of boron has been attributed by some authors to the contribution of boron to leaf photosynthesis, cell elongation and division, and the metabolism of nitrate [45]. This is associated with the improvement of plant cell wall and membrane stability, resulting in a larger leaf area, which in turn, exerts a significant influence on overall plant growth [11]. Wang *et al.* [21] observed that the root system of young peach trees treated with nano-carbon materials exhibited enhanced growth. This was accompanied by an increase in the net photosynthetic rate and chlorophyll content. A comparison of the two rGO concentrations revealed that the concentration of 6 g/L resulted in the tallest plants. Anjum *et al.* [46] studied the effect of graphene oxide sheets on seed germination and seedling growth of *Vicia faba* and found that moderate concentrations, specifically 400 and 800 mg/L, enhanced plant growth, whereas higher (1600 mg/L) and lower (100 and 200 mg/L) concentrations has a detrimental effect. In addition to being safe, graphene nanoplatelets improved the growth and yield of *Capsicum annuum* and *Solanum melongena* as demonstrated by [18]. Zhou *et al.* [47] stated that graphene oxide at 80 mg L^{-1} showed positive effects on *Iris pseudacorus* growth through promoting nutrient uptake and photosynthesis performance.

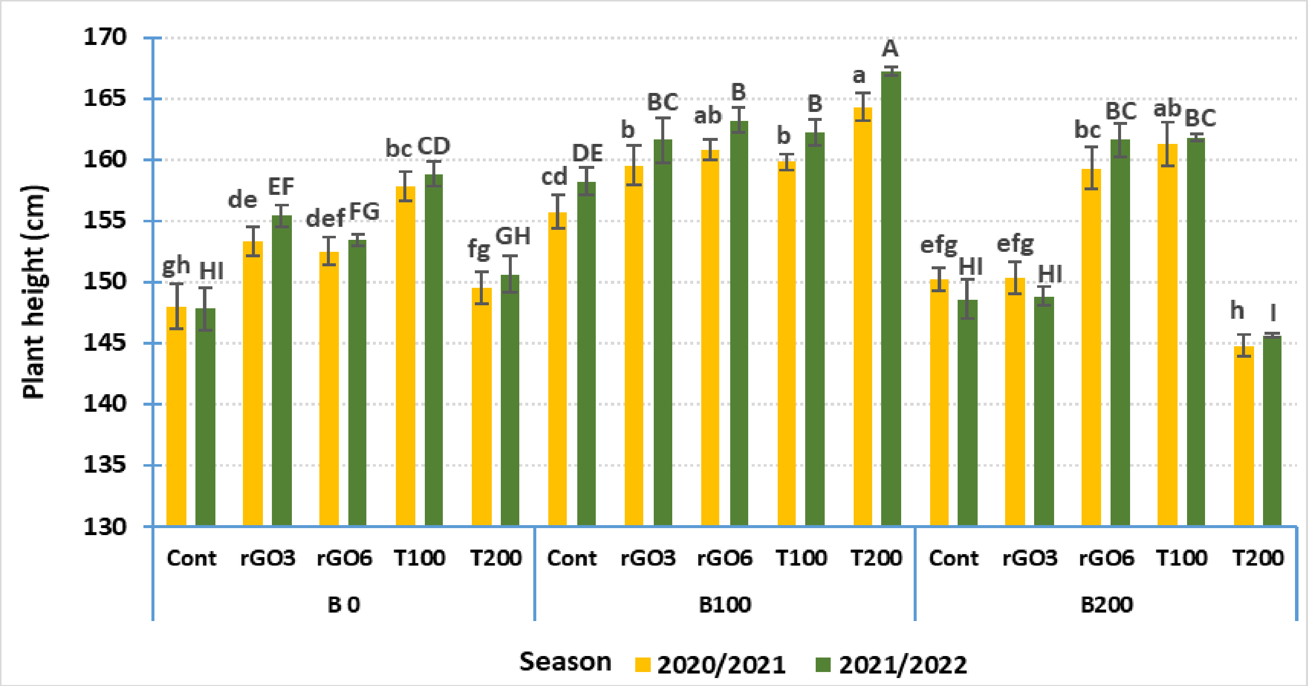

Figure 1: Effect of boron (B) at 0, 100 and 200 ppm, reduced graphene oxide (rGO) at 3 and 6 g/L and tryptophan (T) at 100 and 200 ppm as foliar treatments (mean ±SE) on plant height (cm) of *F. vulgare* plants. Different lowercase letters indicate significant differences among different treatments within 2020/2021 season, and different uppercase letters indicate significant differences among different treatments within 2021/2022 season at p=0.05 using LSD test.

Figure 2: Non-treated control *F. vulgare* plants (A) compared with those treated with the combination of boron at 100 ppm with tryptophan at 200 ppm (B) or reduced graphene oxide (rGO) at 6 g/L (C), bar=10 cm.

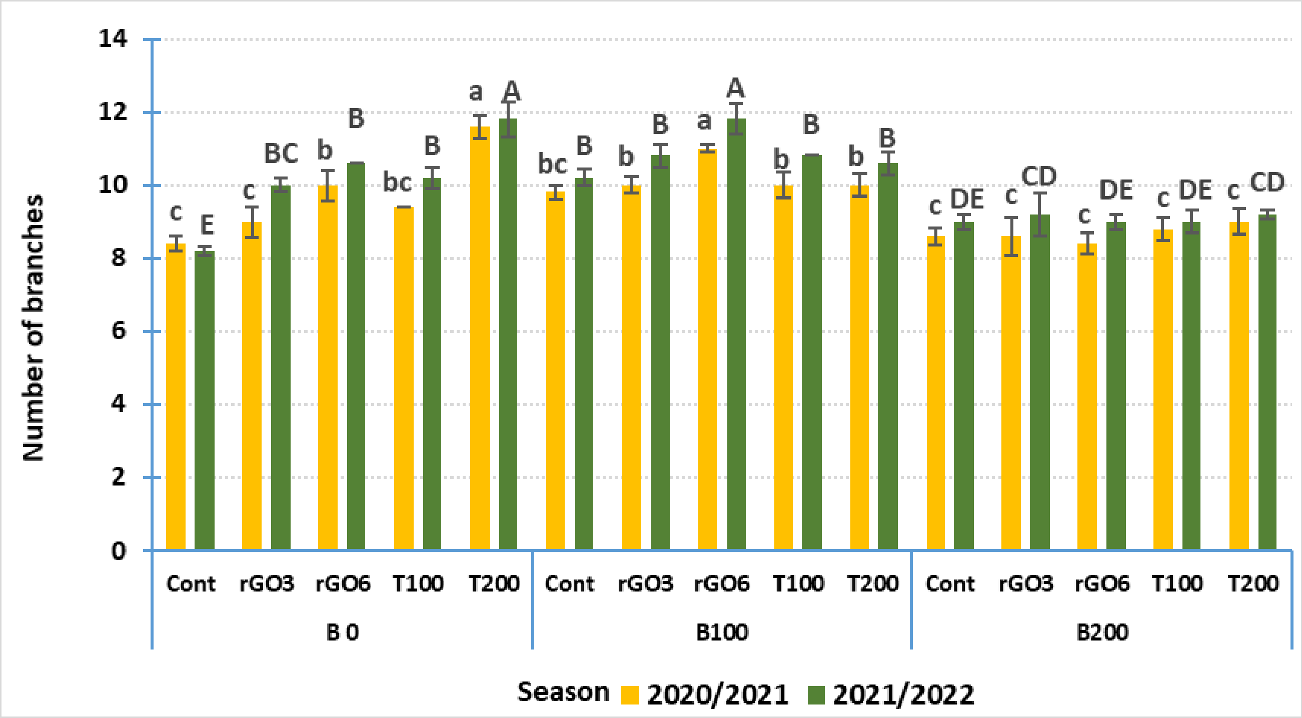

Figure 3: Effect of boron (B) at 0, 100 and 200 ppm, reduced graphene oxide (rGO) at 3 and 6 g/L and tryptophan (T) at 100 and 200 ppm as foliar treatments (mean ±SE) on number of branches of *F. vulgare* plants. Different lowercase letters indicate significant differences among different treatments within 2020/2021 season, and different uppercase letters indicate significant differences among different treatments within 2021/2022 season at p=0.05 using LSD test.

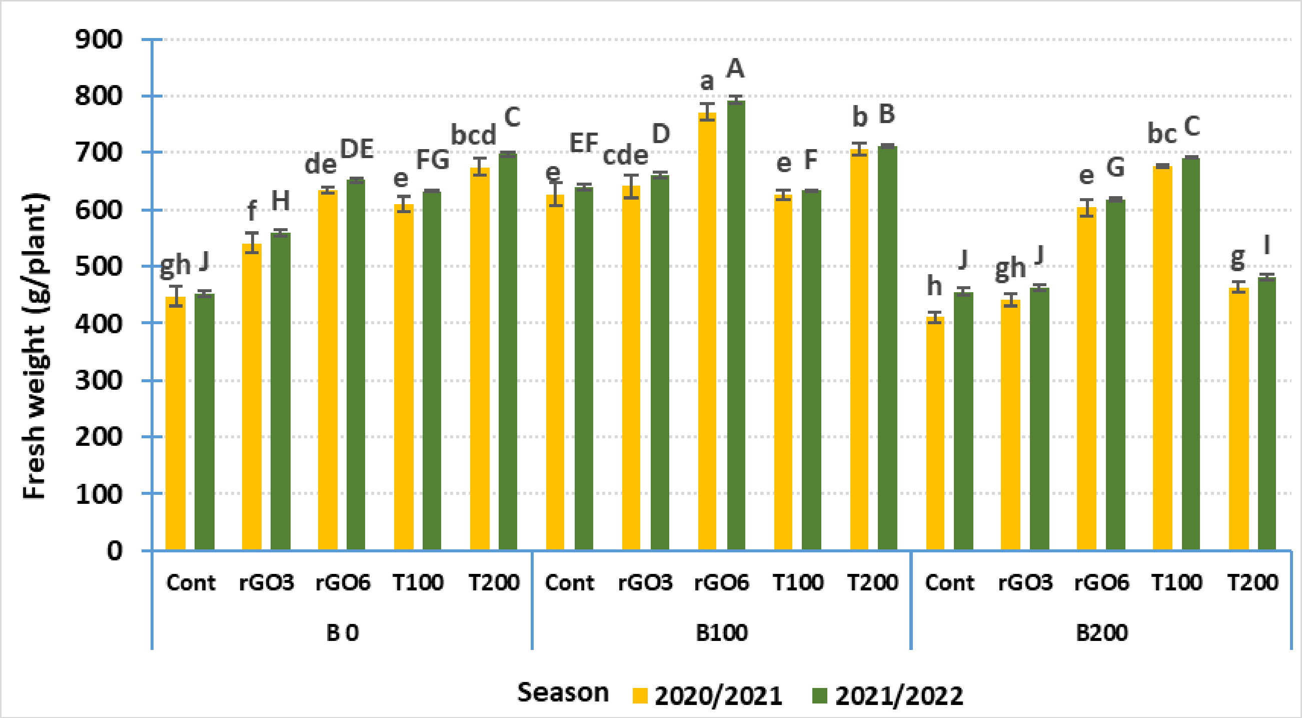

Figure 4: Effect of boron (B) at 0, 100 and 200 ppm, reduced graphene oxide (rGO) at 3 and 6 g/L and tryptophan (T) at 100 and 200 ppm as foliar treatments (means ± SE) on fresh weight (g/plant) of fennel plants. Different lowercase letters indicate significant differences among different treatments within 2020/2021 season, and different uppercase letters indicate significant differences among different treatments within 2021/2022 season at p=0.05 using LSD test.

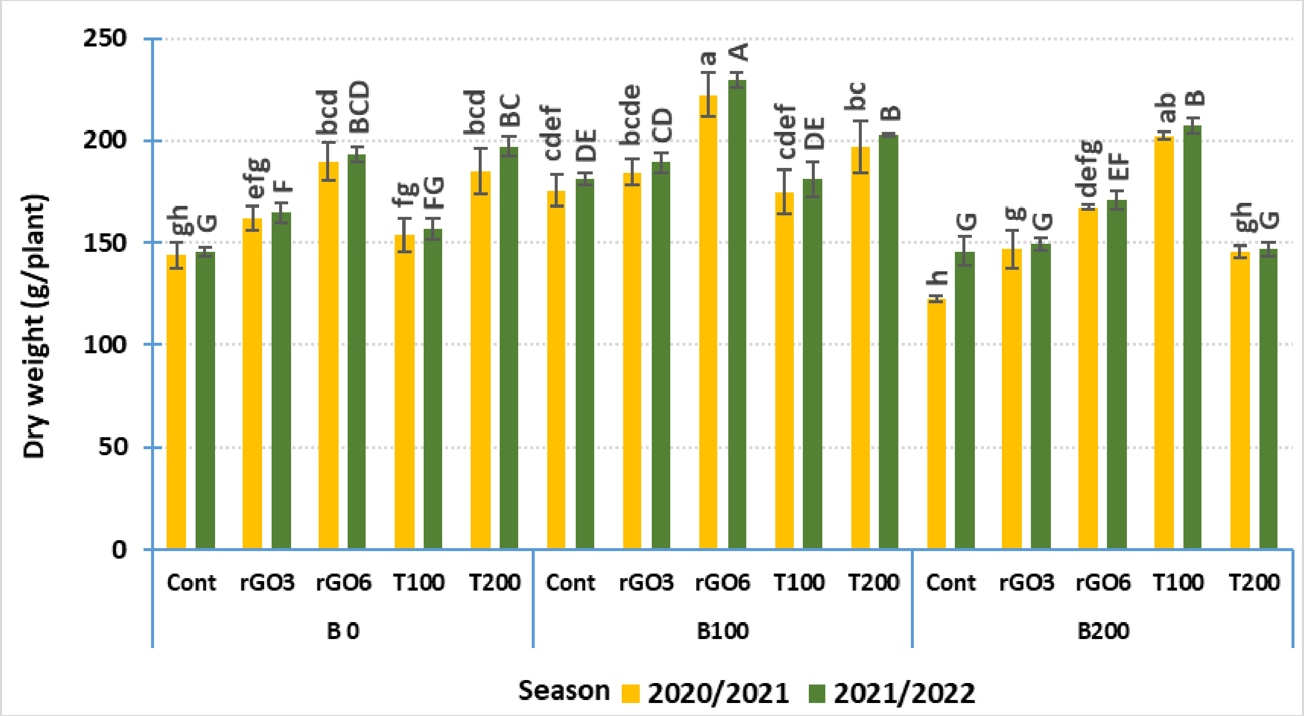

Figure 5: Effect of boron (B) at 0, 100 and 200 ppm, reduced graphene oxide (rGO) at 3 and 6 g/L and tryptophan (T) at 100 and 200 ppm as foliar treatments (mean ±SE) on dry weight (g/plant) of *F. vulgare* plants. Different lowercase letters indicate significant differences among different treatments within 2020/2021 season, and different uppercase letters indicate significant differences among different treatments within 2021/2022 season at p=0.05 using LSD test.

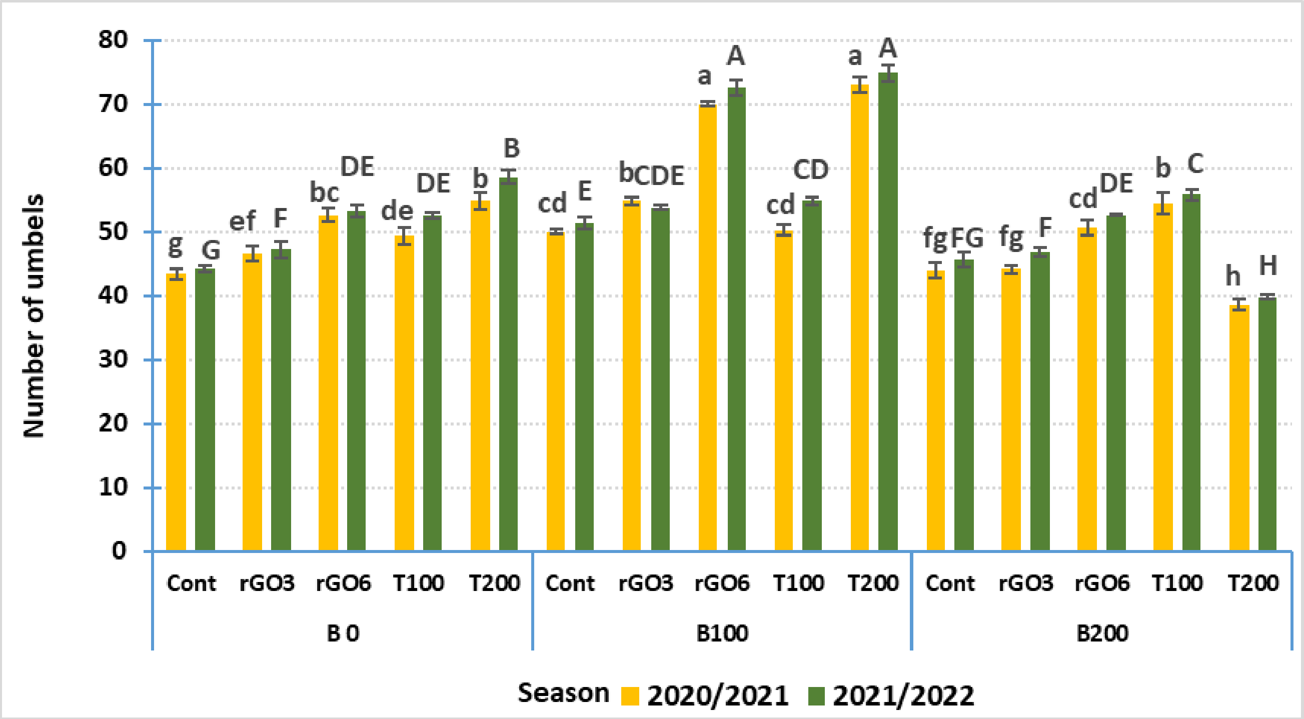

Figure 6: Effect of boron (B) at 0, 100 and 200 ppm, reduced graphene oxide (rGO) at 3 and 6 g/L and tryptophan (T) at 100 and 200 ppm as foliar treatments (mean ±SE) on number of umbels of *F. vulgare* plants. Different lowercase letters indicate significant differences among different treatments within 2020/2021 season, and different uppercase letters indicate significant differences among different treatments within 2021/2022 season at p=0.05 using LSD test.

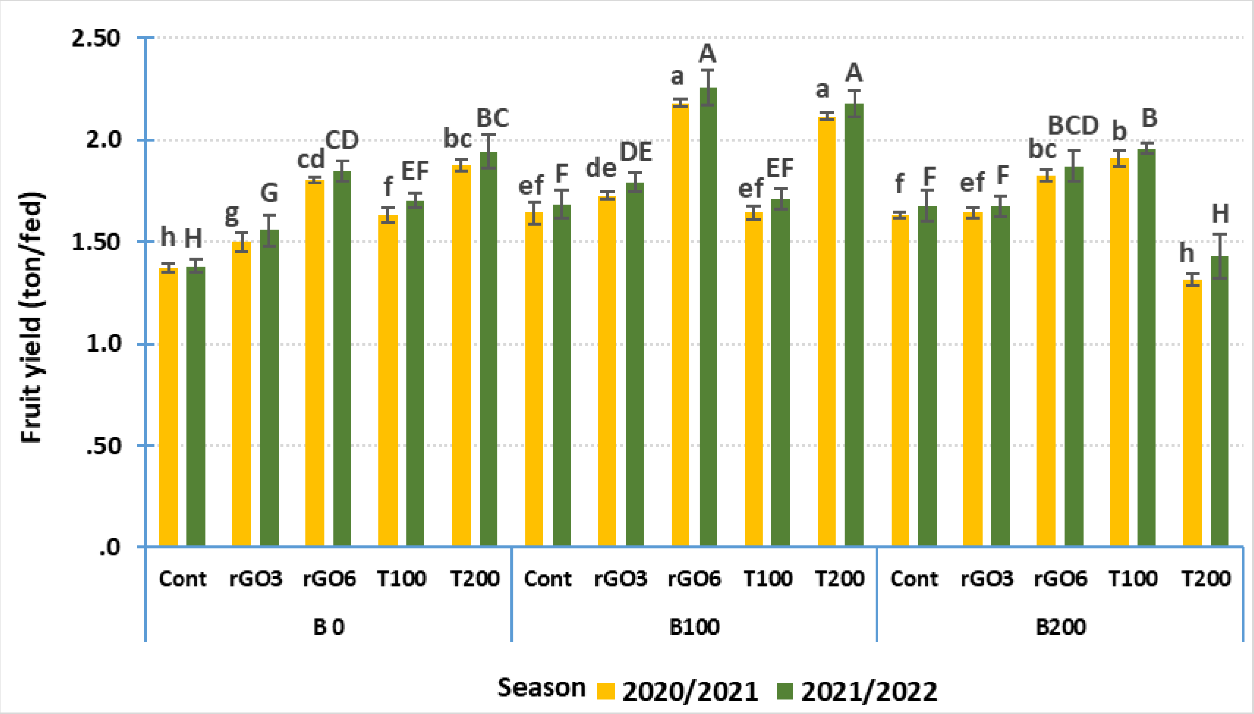

Figure 7: Effect of boron (B) at 0, 100 and 200 ppm, reduced graphene oxide (rGO) at 3 and 6 g/L and tryptophan (T) at 100 and 200 ppm as foliar treatments (mean ±SE) on fruit yield (ton/fed) of *F. vulgare* plants. Different lowercase letters indicate significant differences among different treatments within 2020/2021 season, and different uppercase letters indicate significant differences among different treatments within 2021/2022 season at p=0.05 using LSD test.

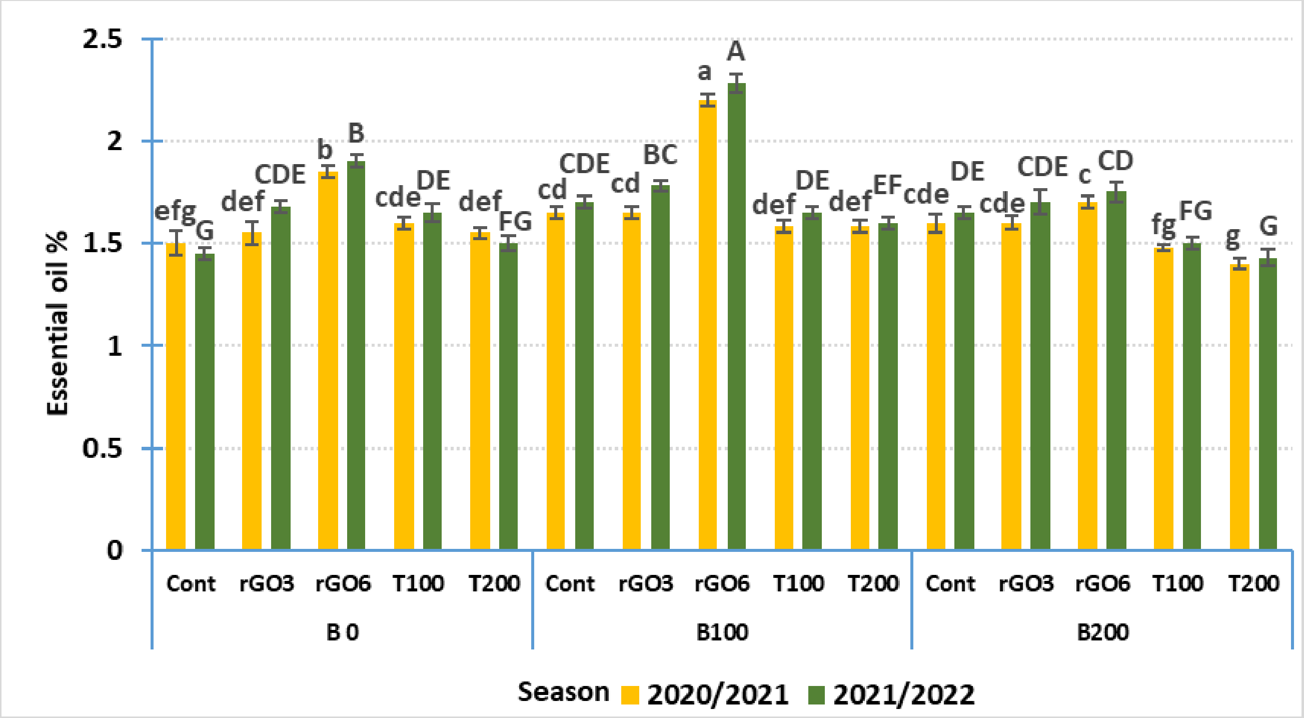

Figure 8: Effect of boron (B) at 0, 100 and 200 ppm, reduced graphene oxide (rGO) at 3 and 6 g/L and tryptophan (T) at 100 and 200 ppm as foliar treatments (mean ±SE) on essential oil % of *F. vulgare* plants. Different lowercase letters indicate significant differences among different treatments within 2020/2021 season, and different uppercase letters indicate significant differences among different treatments within 2021/2022 season at p=0.05 using LSD test.

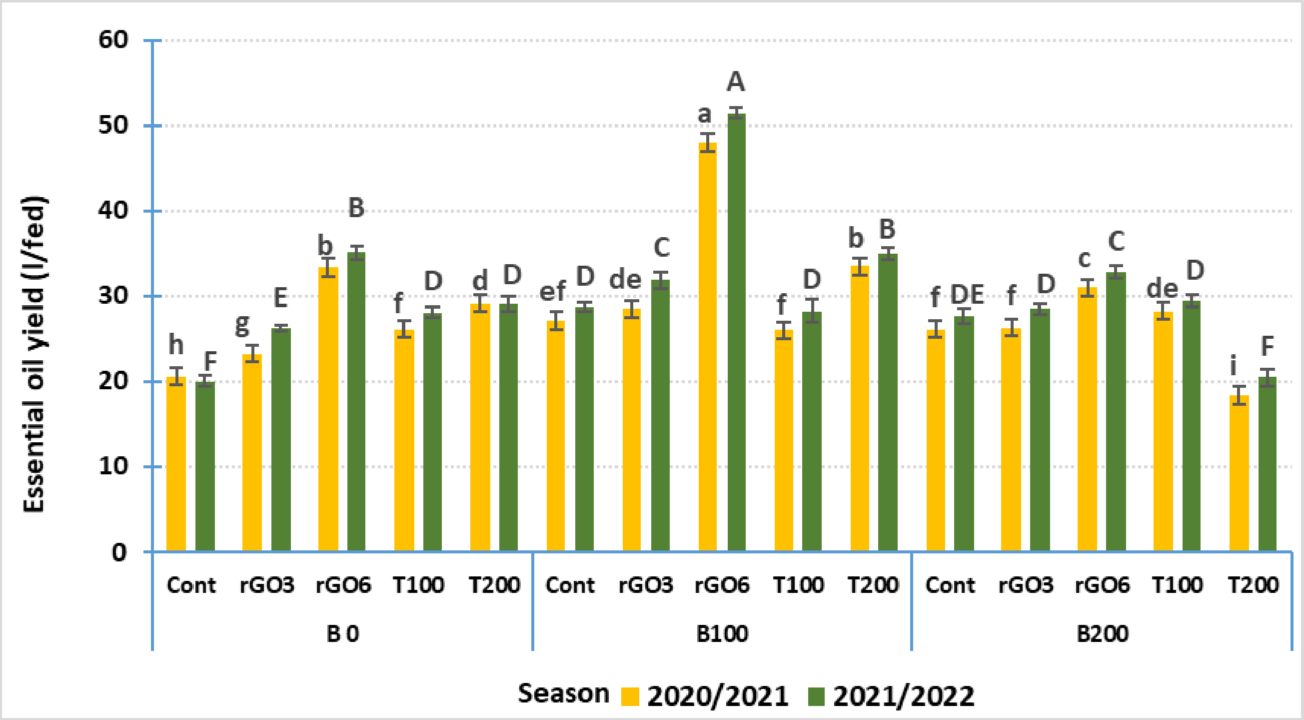

Figure 9: Effect of boron (B) at 0, 100 and 200 ppm, reduced graphene oxide (rGO) at 3 and 6 g/L and tryptophan (T) at 100 and 200 ppm as foliar treatments (mean ±SE) on essential oil yield (l/fed) of *F. vulgare* plants. Different lowercase letters indicate significant differences among different treatments within 2020/2021 season, and different uppercase letters indicate significant differences among different treatments within 2021/2022 season at p=0.05 using LSD test.

Table 1: The comparison of the essential oil composition in *F. vulgare* seeds as affected by the combined treatments of boron (B) at 100 ppm with reduced graphene oxide (rGO) at 6 g/L or tryptophan (T) at 200 ppm compared with the control (non-treated)

RT[1]	Components	Peak area %			MW[2]	MF[3]	CAS no
		Control	rGO 6 g/L + B 100 ppm	T 200 ppm + B 100 ppm			
4.29	Sabinene	0.37	0.30	0.37	136	$C_{10}H_{16}$	3387-41-5
4.59	β-Pinene	0.37	0.36	0.33	136	$C_{10}H_{16}$	127-91-3
5.13	p-Cymene	0.33	0.21	0.24	134	$C_{10}H_{14}$	99-87-6
5.32	Limonene	17.58	17.80	21.37	136	$C_{10}H_{16}$	138-86-3
5.45	cis-Ocimene	0.81	2.10	1.64	136	$C_{10}H_{16}$	6874-10-8
5.89	γ-Terpinene	0.55	0.24	0.43	136	$C_{10}H_{16}$	99-85-4
6.26	Fenchone	6.66	6.43	2.38	152	$C_{10}H_{16}O$	1195-79-5
7.42	Camphor	-	0.42	-	152	$C_{10}H_{16}O$	76-22-2
8.84	Estragole	66.52	66.68	67.76	148	$C_{10}H_{12}O$	140-67-0
10.96	Anethole	0.59	0.56	0.53	148	$C_{10}H_{12}O$	104-46-1
16.07	α-copaene	-	-	0.25	204	$C_{15}H_{24}$	NA
18.86	Apiol	0.36	-	-	222	$C_{12}H_{14}O_4$	523-80-8
26.42	n-Hexadecanoic acid	2.39	2.18	2.25	256	$C_{16}H_{32}O_2$	57-10-3
29.41	Linoelaidic acid	1.55	1.11	1.19	280	$C_{18}H_{32}O_2$	506-21-8
29.58	Oleic acid	1.34	1.62	1.26	282	$C_{18}H_{34}O_2$	112-80-1
37.94	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	0.56	-	-	356	$C_{21}H_{40}O_4$	3443-84-3

[1]Retention time, [2]Molecular weight, [3] Molecular formulae, rGO=reduced graphene oxide, B =boron, T= tryptophan

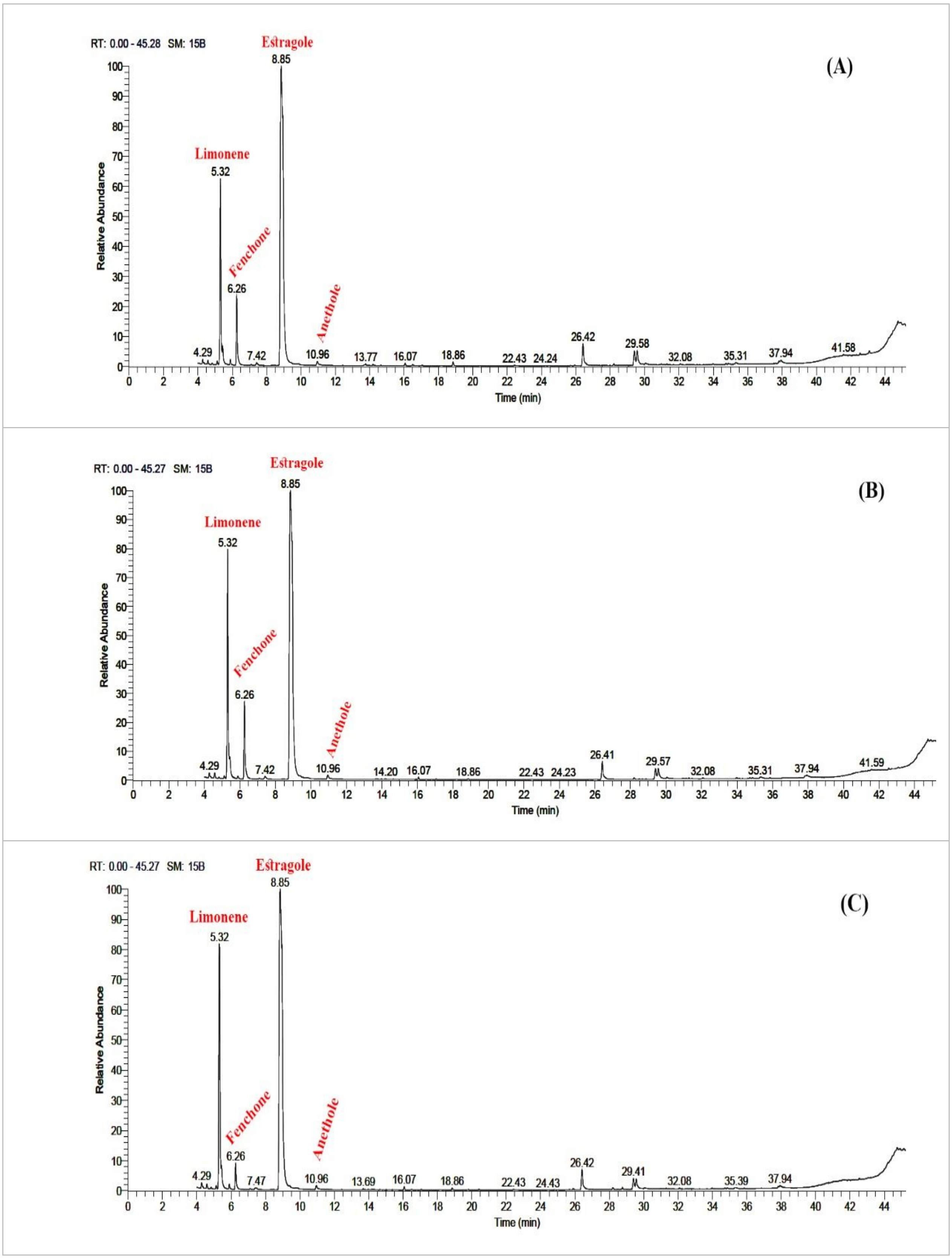

Figure 10: Chromatograms derived from GC-MS analysis of the essential oil in *F. vulgare* seeds. A: the control (non-treated) treatment, B: boron at 100 ppm combined with reduced graphene oxide material at 6 g/L, C: boron at 100 ppm combined with tryptophan at 200 ppm.

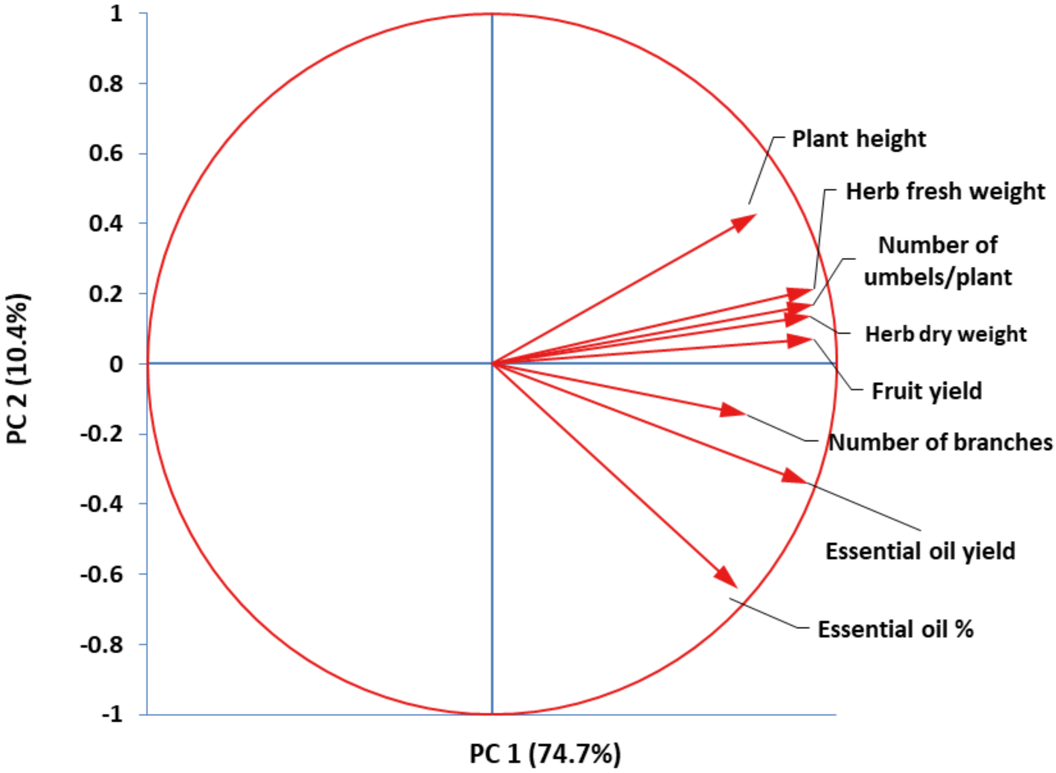

Figure 11: Correlation biplot of principal components (PC1 and PC2) of *F. vulgare* plant characteristics as affected by boron, reduced graphene oxide and tryptophan application on growth and productivity of *F. vulgare* plant (average of the two growing seasons).

Recently, [23] found that reduced graphene oxide treatments at 30 mg/L significantly enhanced wheat plants under Pb stress. This was achieved by increasing the chlorophyll content as well as antioxidant enzyme activities and reducing the Pb uptake. These previous results are consistent with those of several previous studies which have demonstrated the positive effect of tryptophan at doses of 50 and 100 ppm on vegetative growth of a number of species, including *Foeniculum vulgare* [34,35], *Philodendron erubescens* [48] and *Rosmarinus officinalis* [36]. The increase in plant growth as a result of tryptophan application may be due to its conversion to IAA [49]. The converted IAA plays an important role in activating plant growth, consequently the plant height, number of branches/plant and plant dry weight could be increased. Additionally, it influences plant growth by participating in the biosynthesis of various natural products, including phytoalexins and phenylpropanoid [32]. In response to insufficient levels of endogenously produced auxin, plants react to exogenously applied tryptophan [33].

3.2. Yield attributes

Changes in the number of umbels per plant and fruit yield per feddan (0.42 ha) in both growing seasons were found to be significantly influenced by both the type and concentration of the supplemented substances. According to the results presented in Fig. 6, the application of 100 ppm of boron resulted in a higher number of umbels per plant compared to the control (non-treated plants) and plants treated with a higher concentration of boron (200 ppm). Also, the application of tryptophan or reduced graphene oxide (rGO) resulted in an increase in the number of umbels per plant in both growing seasons compared to the control, with significant differences only in plots treated with 100 or 200 ppm of tryptophan and 6 g/L of rGO. The *F. vulgare* plants that were treated with the combination of boron at 100 ppm and tryptophan at 200 ppm had the highest number of umbels per plant (73.0 and 74.8), followed by the plants treated with boron at 100 ppm and rGO at 6 g/L (70.0 and 72.6).

When comparing the general effect of boron treatments on fruit yield, the plants treated with boron produced a higher yield than the control (Fig. 7). When comparing the effect of rGO and tryptophan treatments, fruit yield was significantly higher in plants treated with rGO at 6 g/L, tryptophan at 200 ppm, compared to those treated with any of the other rGO and tryptophan treatments. The application of boron at 100 ppm in combination with rGO at 6 g/L resulted in the highest increase in fruit yield, followed by the combined treatment of boron at 100 ppm with tryptophan at 200 ppm.

The results of our study showed that boron has the potential to interfere with the flowering, fruit set and essential oil yield of plants. This interference is strongly related to the success and quality of pollination as suggested by [9] and [10]. When fennel plants were sprayed with boron at 40 and 80 ppm, there was a significant improvement in yield parameters [40]. Mheidi *et al.* [50] demonstrated that anise plants sprayed with boron at a rate of 1.5 kg/ha exhibited a significant increase compared to untreated plants, with an increase of 20.4% in the number of inflorescences, 30.8% in fruit yield per plant. Abdallah and coauthors [41] found that the foliar application of boron at a concentration of 150 ppm led to a significant improvement in the growth of coriander plants compared to untreated plants. This resulted in a 20% increase in fruit yield per plant and about a three-fold increase in essential oil yield per plant. Several other crops such as olive, highbush blueberry, barley, wheat, tomato and *Arabidopsis thaliana* have also shown better fruit growth and yield following boron application, with increased fruit and seed set leading to higher production [42,43,44,7]. The foliar application of boron at 200 mg/L on olive trees led to the maximum increase in fruit yield (275%) and oil yield (318%) [42]. Similarly, the application of the same concentration of boron (200 mg/L) on highbush blueberry cultivar Brigitta led to increased fruit set and soluble solids, in comparison to higher concentrations (400 and 800 mg/L) [43]. This is consistent with the findings of previous reports, such as those of [51], who observed an increase in the number of flowers and fruit in tomato plants in response to carbon nanotubes (CNTs) application at concentrations of 50 and 200 µg/mL. Additionally**,** [52] confirmed the positive effect of NCM at a concentration of 3 g/L on the number of inflorescences per spike and the weight of new gladiolus tubers when compared to higher concentrations of NCM (5 or 7 g/L). Furthermore, the application of liquid nano-carbon was found to significantly increase the annual yield of Chinese cabbage [53]. Similarly, the application of fullerol at 0.943–47.2 nM led to increased fruit weight, number, and length in bitter melon [54]. The application of rGO resulted in modifications to the number of umbels per plant and fruit yield in addition to the modulation of plant growth. The higher concentration of 6 g/L was found to elicit significantly more favorable effects than 3 g/L. Zhao *et al.* [55], showed that graphene oxide at a concentration ranging from 10 to 1000 mg/L had no significant effect on flowering of *Arabidopsis thaliana*. Additionally, [24] reported the absence of phytotoxicity in tomato and pepper plants treated with reduced graphene oxide (1, 10, 100 mg/L), accompanied by favourable effects on photosynthetic pigment accumulation, flowering and plant height as well as dry weight. Earlier reports have documented the stimulative effect of tryptophan on fruit yield and productivity of plants. El-Awadi and Hassan [35] reported a significant increase in the number of umbels, and the weight of seeds of *F. vulgare* treated with tryptophan at a concentration of 100 mg/L, in comparison to the higher concentration of 500 g/L and the control. Similar results were reported by [34], where the application of tryptophan at 100 mg/L led to a significant increase in the flowering and fruit of *F. vulgare* plants. The exogenous application of tryptophan also led to improvements in fruit in a number of other species, including *Crinum asiaticum* [56], *Iberis amara* [57] and *Rosmarinus officinalis* [36].

3.3. Essential oil percentage and yield

The content of essential oils varied significantly between *F. vulgare* plants exposed to different boron concentrations. The application of boron in a lower concentration (100 ppm) led to higher essential oil content, with levels of 1.65 and 1.70% of d.w., in the 2020/2021 and 2021/2022 seasons, respectively, compared to control plants (Fig. 8). In contrast, the application of boron at a concentration of 200 ppm did not cause any significant changes. When comparing the overall effect of rGO and tryptophan treatments in the two growing seasons, it can be seen that rGO at 6 g/L had the best effect. It caused a significant increase in the percentage of oil content from 1.50 and 1.45% recorded for the control to 1.85 and 1.90%. The results of the interaction between boron, rGO and tryptophan treatments showed significant variations at $p \leq 0.05$. It was noted that the application of boron at 100 ppm in combination with rGO at 6 g/L resulted in the highest content of essential oils (2.20 and 2.28% d.w.) in the 2020/2021 and 2021/2022 seasons, respectively.

As the percentage of essential oil and fruit yield increased, the amount of essential oil yield also increased, with the highest value (47.99 and 51.44 l/fed. in the 2020/2021 and 2021/2022 seasons, respectively) occurring on the plants treated with boron at 100 ppm combined with rGO at 6 g/L (Fig 9). The second most favorable treatment was rGO at 6 g/l and a combination of boron at 100 ppm and tryptophan at 200 ppm.

The results of our study showed that boron has the potential to interfere with essential oil yield of plants. This interference is strongly related to the success and quality of pollination as suggested by [9] and [10]. When fennel plants were sprayed with boron at 40 and 80 ppm, there was a significant improvement in yield parameters [40]**.** Mheidi *et al.* [50] demonstrated that anise plants sprayed with boron at a rate of 1.5 kg/ha exhibited a significant increase compared to untreated plants, with an increase of 9% in essential oil content per plant. Karayel [58] found that different boron doses (0, 1 and 8 liters per decare [1000 square meters]) affected *F. vulgare* oil content. The 8-litre dose was found to induce the highest oil content (3.43%). Abdallah and coauthors [41] found that the foliar application of boron at a concentration of 150 ppm led to a significant improvement in the growth of coriander plants compared to untreated plants. This resulted in a 20% increase in fruit yield per plant and about a three-fold increase in essential oil yield per plant. The foliar application of boron at 200 mg/L on olive trees led to the maximum increase in oil yield (318%) [42]. Earlier reports have documented the stimulative effect of tryptophan on oil productivity of plants. Results were reported by Abdel-Rahman *et al.* [34], where the application of tryptophan at 100 mg/L led to a significant increase in oil yield of *F. vulgare* plants. The exogenous application of tryptophan also led to improvements in fruit and oil productivity in a number of other species, including *Crinum asiaticum* [56], *Iberis amara* [57] and *Rosmarinus officinalis* [36].

3.4. Composition of essential by GC-MS analysis

The analysis of the phytocomponents in *F. vulgare* essential oil was conducted using GC-MS for the control treatment and two treatments that induced superior agronomic traits in the treated plants: boron at 100 ppm plus rGO at 6 g/L, and boron at 100 ppm plus tryptophan at 200 ppm. The composition and characteristics of the detected components can be found in Table 1, and a diagram illustrating the derived chromatograms is presented in Fig. 10. The content of estragole (67.76%) and limonene (21.37%)

increased as a result of foliar spraying plants with tryptophan at 200 ppm plus boron at 100 ppm (Fig. 10 C), while fenchone (2.38%) and anethole (0.53%) decreased in comparison with their concentrations in the control plants (Fig. 10. A). However, rGO at 6 g/L plus boron at 100 ppm did not cause any noticeable differences in the content of the detected components compared to the control (Fig. 10. B). Two compounds were detected in the oil of the control treatment: apiol (0.36%) and 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester (0.56%), but these compounds were not present in the essential oil of *F. vulgare* plants sprayed with rGO at 6 g/L plus boron at 100 ppm or tryptophan at 200 ppm plus boron at 100 ppm. Additionally, α-copaene was only detected in plants treated with rGO 6g plus boron at 100 ppm.

3.5. Principal component analysis (PCA) of the measured traits

The first two principal components, PC1 and PC2, together explained a variance of 74.7% of the total variance as shown in the PCA biplot in Fig. 11. PC1 showed an intermediate positive loading for all the recorded traits as indicated by the direction of the vectors, suggesting that these parameters contribute significantly to PC1. All the vectors are clustered together, indicating a positive correlation between them. Moreover, the highest positive correlation was observed between fresh and dry weights, as well as between number of umbels per plant and fruit yield. PC2 showed a high positive loading for plant height, but a negative loading for the number of branches, essential oil percentage and yield. It can be concluded that the fresh and dry weights of *F. vulgare* are strong determinants of fruit yield and thus essential oil yield. Therefore, the values of PC1 and PC2 can be used to analyze the performance of *F. vulgare* plants that have been treated with similar substances.

The PCA results obtained in this study could clarify and interpret the response of F. vulgare plants to the treatments applied. As showed by the PCA, fruit yield was highly correlated with the number of umbels per plant as well as fresh and dry weights. This suggests a direct/indirect causal effect of plant mass and number of umbels on fruit yield. The highest fruit yield was recorded for *F. vulgare* when treated with boron at 100 ppm combined with rGO at 6 g/L. This is the same treatment that resulted in the highest number of branches per plant. It was also evident that the biomass of *F. vulgare* made a significant contribution to the fruit yield, with the application of boron at 100 ppm and rGO at 6g/L, resulting in the highest fresh and dry weights and, consequently, an improvement in fruit yield. The correlation between the growth of *F. vulgare* and the yield components has been previously reported in literature. For example, studies conducted by [59,60,61], demonstrated a positive correlation between *F. vulgare* seed yield and plant growth parameters including plant height, number of branches, and number of umbels per plant.

The interactions of boron with other components or mineral elements are complex, and the effects that result can be either antagonistic or synergistic. The results of our study, demonstrated that the applied supplements exhibited synergistic effects. The combined application of boron and reduced graphene oxide resulted in a significant increase in vegetative growth, flowering and fruit yield in *F. vulgare*. According to Wang [21], NCM (nano-carbon materials) effectively adsorbs minerals in the soil, thereby reducing the rate of mineral loss and improving their utilization by plants. In addition, NCM facilitates the controlled and gradual release of micronutrients [62]. Given the positive effects of NCM on plant growth and their synergistic effects with fertilizers, including micronutrients, there has been a growing interest in combining them with fertilizers to produce so-called nanocarbon-enhanced fertilizers or the nanocarbon synergist. The combined application of boron and tryptophan, resulted in a significant increase in essential oil yield, accompanied by enhanced growth and yield of *F. vulgare* plants. The principal constituent of the seed essential oil under investigation was estragole (=methyl chavicol), a finding that is consistent with those of previous studies [63,64]. The synergistic effect of boron and amino acids has been previously reported to enhance the growth and yield of *Vigna unguiculata* L. [65] and the yield of pods and seeds of *Pisum sativum* L [66].

4. Conclusions

Based on two seasons of field trials, it can be concluded that the foliar application of boron in combination with reduced graphene oxide or tryptophan shows a synergistic and positive response, by improving the growth and productivity of *F. vulgare* plants. The combined treatment of boron at 100 ppm with either reduced graphene oxide at 6 g/L or tryptophan at 200 ppm resulted in the tallest plants and the highest production of fresh and dry weights, as well as the highest number of branches, which was reflected in the number of umbels and fruit yield. The yield of fruit was highly correlated with the number of umbels/plants, as well as with the fresh and dry weights, as confirmed by PCA analysis. The combination of boron with tryptophan was found to result in a higher oil yield than that achieved with reduced graphene oxide. It is therefore recommended that, in order to achieve enhanced growth and fruit yield in *F. vulgare*, the use of combined treatments (boron at 100 ppm with either reduced graphene oxide at 6 g/L or tryptophan at 200 ppm) should be considered. Conversely, when the objective is to enhance essential oil yields, the use of boron at 100 ppm in combination with tryptophan at 200 ppm is advised. The findings of this study suggest new avenues for sustainable production of aromatic and medicinal herbaceous crops through the investigation of the potential of amino acids, nano-carbon compounds, and micronutrients to enhance their growth and productivity.

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