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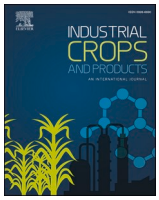

A new method in mitigation of drought stress by chitosan-coated iron oxide nanoparticles and growth stimulant in peppermint

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ABSTRACT

Damage to the plant's photosynthetic system is known as the main symptoms of sensitivity to environmental stresses. Little is known about beneficial role of new chitosan compounds such as Kitoplus®, and chitosan-coated nanomaterials on alleviation of adveres effects of drought stress in plants. In the present study, a factorial experiment was conducted in a split-plot manner based on a randomized complete block design (RCBD) with three replications to evaluate the effects of Kitoplus® growth stimulant and Chitosan-Coated Iron Oxide Nanoparticles (Fe-CTs NPS) on peppermint growth, chlorophyll fluorescence and essential oil biosynthesis under drought stress conditions. Treatments included three levels of drought stress (irrigation at soil moisture levels of 30%, 60%, and 90%), three concentrations of Kitoplus®(control without Kitoplus®, 0.5% and 1%), and three concentrations of Fe-CTs NPs (control without Fe-CTs NPs, 5 and 10 μM). Drought stress, growth stimulation treatment with Kitoplus®, and Fe-CTs NPs had significant dual and triple effects on the studied physiological traits. According to the results, chlorophyll index and stomatal conductance were significantly lower in control plants than in those undergoing drought stress. Under 60% field capacity moisture conditions, the chlorophyll index was increased with 1% Kitoplus® treatment. The maximum fluorescence (Fm) and variable fluorescence (Fv) raised as the drought stress intensity increased. The highest Fm and Fv were observed in plants treated with 1% Kitoplus® and 10 μM Fe-CTs NPS under 30% soil moisture stress. Results also showed that the highest value of menthone (33.31%) was obtained in plants treated with Kitoplus® at 1% and Fe-CTs NPs at 10 μM under drought stress of 60% field capacity. In addition, the highest amount of menthol (26.5%) was observed in plants treated with 0.5% Kitoplus® under drought stress of 30% field capacity. The overall importance of the study lies in devising the simultaneous application of biostimulants and nanomaterials in enhancing essential oils production in the mint plant under drought stress challenges.

1. Introduction

Peppermint (Mentha piperita L.) is one of the most important medicinal plants belonging to the Lamiaceae family (Bupesh et al., 2007). The plant is native to temperate regions, particularly in Europe, North America, and North Africa, and is cultivated worldwide (Singh et al., 2011). The essential oil content of this species ranges from 0.1- to 0.5% and includes menthol 28-42.2%, menthon 18-28% menthone 18-24 menthofuran 19.8%, and methyl acetate 3–10% (McKay and Blumberg, 2006; Schmidt et al., 2009). Menthol and menthone are the main components of essential oils, exhibiting antimicrobial properties. The amount of menthol is the main criterion used in the determination of peppermint essential oil quality (Kumar et al., 2004).

Agricultural production facing various threats include both abiotic and biotic stresses would have more adverse effects in the future, consequently, enhancing growth and productivity considered the major

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goal for the scientist to reached food security. Moreover, increasing global temperature leads to a decrease in agricultural land due to a lack of sufficient water for plant cultivation. It is considered the greatest threat to humanity and living organisms (Kazemi and Ghorbanpour, 2017; Mirajkar et al., 2019). The effect of drought stress on plant growth and productivity depends on several factors, including plant genotype, duration of stress, other environmental conditions, and the plant's developmental stage during stress (Mateja et al., 2007; Li et al., 2015). Drought stress affects the plant at molecular levels causing changes in the expression of stress-responsive genes, growth retardation, and accumulation of organic matter

One of the main symptoms of plant sensitivity to environmental stresses is damage to the photosynthetic system. The amount of chlorophyll and photosynthesis efficiency in leaves under stress decreases due to the destruction of proteins and lipid components of the thylakoid membrane (Hu et al., 2006). Photosystem II is the first part of the photosynthetic apparatus that responds to stresses (Baker, 1991; Hasanuzzaman et al., 2013). Indeed, photosystem II is more sensitive to environmental stresses than photosystem I in the photosynthetic electron transport chain. One of the reasons for this greater sensitivity is the presence of a water decomposing complex in the photosystem II (Apostolova et al., 2006). The intensity of chlorophyll fluorescence may exhibit the plant's ability to withstand environmental stresses, the health of the thylakoid membrane, the relative efficiency, and the rate of electron transfer from photosystem I to photosystem II, as well as damage caused by stress in plants (Zlatev, 2009).

Today, nanotechnology is widely used in modern agriculture to realize the concept of precision agriculture. Nanotechnology uses nanoparticles with a size between 1 and 100 nanometers (Hatami et al., 2016). Nanoparticles (NPs) are used to mitigate drought stresses in plants, improve nutrition and improve field management because of their unique characteristics such as ultra small size, high surface-to-volume ratio, and presence of chemical/biochemical moieties on surface, *etc* (Duhan et al., 2017; Fahimirad et al., 2019).

One of such growth stimulants is chitosan (CTs). It is a non-toxic substance, a biopolymer, a nitrogenous and biodegradable polysaccharide obtained by processing the chitin shells of aquatic crustaceans such as lobsters, crabs, shrimps, and other organisms like insects. When applied in plant cultivation, chitosan can alleviate the impacts of environmental stresses such as salinity and drought, leading to improved growth (Limpanavech et al., Wang et al., 2003). In addition, it can be easily modified without affecting its inherent properties compared to other biopolymers. Therefore, CTs have been widely used for various applications by modifying their physicochemical and biophysical properties. CTs-based compounds have recently shown a wide range of antimicrobial and regulatory activities in plants (Malerba and Cerana, 2019).

Malerba and Cerana, 2019) have statedthat metallic-based nanoparticles are biodegradable, very reactionary, and may have unexpected health risks due to perdurability in the food chain. The safety and efficacy of CTs as a base for encapsulating and separating bioactive compounds have been demonstrated by numerous studies. CTs-NPs have been used as carriers for the slow release and uptake of fertilizers, pesticides, herbicides, and plant growth stimulants. Using CTs-NPs to enclose and deliver bioactive compositioncan keep plant cells from the hazardous effects of combustible. Furthermore, CTs-NPs can protect biological molecules from damage caused by temperature, light, and pH. Capsulation in a CTs base as micro or nanoscale carriers has a great potential in agriculture (Mujtaba et al., 2020).

Iron reduces the effects of drought stress. In plants, iron deficiency declines gas exchange, stomatal conductance, water usage efficiency, and increases transpiration (Mazaheri Nia et al., 2010). One of the recent applications of nanotechnology in agriculture is using nano-fertilizers for plant nutrition (Kheirizadeh Arough et al., 2016). The mobility of iron in the soil decreases as soil moisture drops, and due to limited root growth, the plant becomes increasingly deficient in iron. Although plants need iron in small amounts, it is essential for many compounds and physiological processes, such as chlorophyll biosynthesis and the activity of specific enzymes, chloroplast development, light energy reception, and electron transfer from water to $NADP^+$ (Hochmuth, 2011).

Using growth stimulants with an effect on plant development and growth is one of the ways to increase yield per unit area and increase product quality (Radkowski and Radkowska, 2013; Du Jardin, 2015; Karthikeyan Arough et al., 2021). The role of growth stimulants is to increase resistance to stress and accelerate plant development and growth, especially of roots and leaves. Plant growth stimulants improve seed germination and plant biological activity (Salwa and Osama, 2014).

Torabi Giglou et al., (2020) showed that the interaction of water stress and growth stimulation treatment of Kitoplas on the physiological and morphological traits had a significant effect. The same authors showed that the highest amount and percentage of essential oils were observed in plants treated with 10 ppm of Kitoplus®. Growth stimulant Kitoplus® contains chitosan as an active ingredient, which has been used to reduce the effects of drought stress in the present study. In addition, the current study was aimed to investigate the effects of Fe-CTS NPs on mitigation of drought stress negative effects in peppermint. Although, several nanomaterials have applied in agriculture, the use of iron oxide nanoparticles and chitosan coating is a new way of providing the necessary elements to plants. Few studies have been conducted on applying Fe-CTS NPs in medical and aromatic plants, which necessitates research in this field. Therefore, due to the economic importance and increasing demand for mint and its widespread use in various industries as well as knowledge gap on the effects of NPs in this herb under drought, in this study, the role of nanomaterials (Fe-CTsNPs) and plant growth stimulants was assessed with their subsequent evaluation in mitigation of drought stress adverse effects.

2. Materials and methods

2.1. Synthesis of Fe-CTs NPs

To prepare magnetized chitosan, Fe^{2+}/Fe^{3+} ions were first obtained during the co-precipitation process by the in situ method. Briefly, 1 g of chitosan powder (Beijing Be-Better Technology Co., Ltd Beijing, China) was dissolved in 100 mL of 1% (w/w) acetic acid solution and placed at 70 °C. After stirring for 1 h, 2 g of $FeCl_2.4\ H_2O$ and 5.4 g of $FeCl_3.6\ H_2O$ iron salts ($nFe^{3+}/nFe^{2+} = 2$) dissolved in 20 mL of distilled water were added to the chitosan solution. The resulting solution was purified under inert nitrogen gas for half an hour. The pH of the purified solution was then increased to 11 by adding ammonia solution (3 M) dropwise. The dark (Fe^{3+}/Fe^{2+}) loaded chitosan solution showed the formation of Fe_3O_4 nanoparticles. Then it was stirred at 70 °C for one h. Purification of the obtained solution was performed in order to remove unreacted excess material from the reaction medium. After washing several times with additional distilled water, the pH of the solution was 7. To develop a homogeneous solution from the preserved sample, an amount of magnetized chitosan with 100 mL of distilled water was distributed evenly by ultrasonic at a frequency of 50 Hz for 30 min. It was then separated (Fe^{3+}/Fe^{2+}) loaded chitosan by a magnet and dried under a freeze-drying process.

2.2. Plant growth conditions and treatments

This research was carried out as a factorial split-plot experiment based on a randomized complete block design (RCBD) with three replications ($n = 3$). To obtain the field capacity (amount of moisture that remains in the soil after the release of gravity water) moisture percent in the field soil, a plot area of one square meter was irrigated to saturation and covered with plastic. After stopping irrigation and water drainage, soil moisture was measured at 6-hour intervals at a depth of root

development (20 cm) usinga hygrometer. This operation continued until the amount of moisture was almost equal in several consecutive measurements. This moisture percent is considered equal to the moisture of the field capacity. Irrigation treatments are considered based on a percentage of this soil moisture (90%, 60%, and 30%).

The main factor was the irrigation in three soil moisture levels (90%, 60%, and 30% of soil field capacity moisture). Sub-factors were Fe-CTs NPs (Factor A) at three concentrations (control without Fe-CTs NPs, 5 and 10 μM, respectively) and Kitoplus® growth stimulator (Kimia Sabzavar, Iran) at three concentrations (control without Kitoplus®, 0.5% and 1%) (Factor B). Peppermint rhizomes were planted in small pots, kept in the greenhouse for 20 days, and then planted in the main experimental field. Drought stress and foliar applications began 20 days after transferring the peppermint plants to the field. A multi-depth soil sensor device(JXBS-3001-TDR, Weihai JXCT, Ltd. Shandong, China) was used to monitor the moisture to apply drought stress soil. Each block was irrigated independently after soil moisture reached the desired level (90%, 60%, and 30%), with the same volume (400 liters for each block separately after the field capacity moisture was obtained). The Fe-CTs NPs and Kitoplus® were applied (with equal volume for each block, including concentrations specified in the experiment(three times in 15 days intervals. After the last foliar application, traits such as chlorophyll index, stomatal conductance, electrolyte leakage, and proline and chlorophyll fluorescence were evaluated for each sample immediately before the last irrigation. The soil assessment was conducted by sampling in a zigzag pattern from three points at three depths to a depth of 30 cm. The soil samples from root growth depths were sent to the laboratory of the Soil and Water Research Institute, Ardabil, Iran. The soil test results are presented in Table 1.

2.3. Vegetative yield

Fresh weight and dry matter of flowers and leaves were measured to determine yield. Sampling was done twice and randomly from an area of half a meter in the middle of each plot. The plant material was dried at 40 °C for 72 h and weighed for dry matter measurement.

2.4. Chlorophyll index

The chlorophyll index was measured using a SPAD 502 Plus (Spectrum Technologies, Inc. Aurora, IL, USA) chlorophyll meter device.

2.5. Stomatal conductance

The stomatal conductance was measured with Prometer SC-1 (Meter Group, Inc. Washington, USA). For this purpose, 5 plants from each treatment were randomly selected, and one active, fully developed leaf of each plant was used to measure the stomatal conductance so that 30 s after the plant leaf was placed between the clamps of the device, the aperture conductivity was read.Values were normally recorded between 08:30 and 10:30 h, registered within the leaf chamber temperatures of 25.3 ± 0.1 ºC and moisture percentages ranging from 55.7 ± 0.4% to 73.1 ± 0.4%.

Table 1
Results of experimental farm soil test results. Mixed sampling from a depth of 0–30 cm.

Depth (cm)	Clay (%)	Silt (%)	Sand (%)	Acidity (pH)	Salinity (EC_e)	Field Capacity (%)	PWP (%)
0–30	34	40	36	7.59	2.15	26.7	15.96

Salinity (EC_e): the electrical conductivity (EC) of a solution or soil and water mix in the field or laboratory.EC_e is the estimated electrical conductivity of the extract from a saturated soil paste. PWP:permanent wilting point.

2.6. Electrolyte leakage

For this purpose, 15 leaf discs were transferred to tubes with 10 mL of distilled water and placed at room temperature and low light for 24 h. The electrical conductivity of the distilled water with the samples (EC1) was measured by an EC meter (Adwa Instruments, Szeged, Hungary). Then, the test tubes were placed in a water bath (95 °C) for 15 min, and after they had cooled, electrical conductivity was measured again (EC2). Electrolyte leakage was calculated with the following equation (Sairam and Srivastava, 2001):

$$\text{Electrolyte leakage } (\%) = [EC1/EC2] \times 100$$

2.7. Proline

The proline content in leaves was determined using the Bates et al. (1973) method. In brief, 0.5 g of the frozen leaf sample was homogenized in 10 mL of 3% sulfosalicylic acid solution using porcelain mortar. The extract was centrifuged at 4 °C at 12,000 rpm for 10 min. The resulting supernatant was collected in test tubes. To 2 mL of the obtained extract, 2 mL of ninhydrin (2.5%) and 2 mL of glacial acetic acid were added, and the mixture was placed in a water bath at 100 °C for 1 h. Then 4 mL of toluene (10%) were added, and the tubes were placed on a shaker for 15–20 s. Subsequently, the upper (toluene) fraction was removed, and its light absorption was read at 520 nm with a spectrophotometer (HACH Company, Loveland, USA) and compared with proline calibration standard samples.

2.8. Chlorophyll fluorescence

Chlorophyll fluorescence was measured in one fully developed leaf from 5 plants randomly selected from each treatment using a chlorophyll fluorimeter (Hansatech Instruments Ltd. King's Lynn, United Kingdom). First, the clamps of the device were adapted to darkness for about 30 s. Then the diode was connected to the clamp, the clamp valve was opened, and the data was read. The Fv/Fm index was calculated according to the formula. In this formula, Fo is the minimum chlorophyll fluorescence, and Fm is the maximum chlorophyll fluorescence;Fv, the difference between Fm and Fo, is variable fluorescence (Maxwell and Johnson, 2000).Table.2.

2.9. Isolation of essential oils and identification of related compounds

To measure the amount of essential oils, fresh samples of peppermint were dried at 40 °C for 72 h. At the time of essential oil extraction, 30 g of each dry sample was separated, and the essential oil was extracted in the same conditions by Clevenger-type distillation apparatus for 3 h (British Pharmacopoeia, 1993). The essential oil components were identified using gas chromatography device (Model 7890 A, Agilent Company, Santa Clara, America) connected to the mass spectrometer (GC/MS). The capillary column used was HP-5MS with a length of 30 m and a diameter of 0.25 mm. The thickness of the stationary phase was 0.25 μm. The initial temperature was 60 °C with a storage time of 4 min, then 3 °C for 1 min, 100 °C for 2 min, and the temperature was increased 6 °C per min to 270 °C (Adams, 2001).

Table 2
Terms and formulae used in the analysis of the fast chlorophyll a fluorescence.

Term and formulae	Definition
Fo	Fluorescence emitted when all reaction centres (RCs) are open
Fm	Maximum fluorescence emitted when all RCs are closed
Fv = Fm-Fo	Maximum variable fluorescence
Fv/Fm = 1-(Fo/Fm)	Maximum quantum yield of primary photochemistry

2.10. Statistical analysis

Experimental data from a factorial split-plot experiment based on a randomized complete block design were analyzed using ANOVA analysis and Duncan's multiple range tests ($P < 0.05$) by SAS 9.1 for Windows (SAS Institute Inc. Carolina, USA) and SPSS21 for Windows (IBM SPSS Statistics Statistical Procedures Companion, New York, USA) statistical software's test at 5% probability level.

3. Results

3.1. Synthesis and characterization of Fe-CTs NPs

The FT-IR spectrum obtained from pure and magnetic chitosan is shown in (Fig. 1a). According to the chitosan spectrum, the peaks at 3447 cm^{-1} belong to amide groups and (O-H) bonds of adsorbed water. Both peaks at 2920 and 1383 cm^{-1} are related to (C-H) bonds. The (C-O) bond in the chitosan structure was identified with apeak at 1640 cm^{-1}. Based on the magnetic chitosan/Fe_3O_4 spectrum, the peaks observed at 3447, 2924, and 1630 cm^{-1} were particular to chitosan, which have shifted slightly from the original pure chitosan peaks. The peak at 590 cm^{-1} shows the (Fe-O) bond in the magnetic Fe_3O_4 NPs, which is characteristic of magnetic NPs and proves the presence of magnetic nanoparticles in the sample structure. The XRD spectrum shown in (Fig. 1b) corresponds to magnetized chitosan, for which the peaks at $2\theta = 30.3, 35.7, 43.3, 53.8, 57.3$, and 63° are related to (220), (311), (400), (422), (511), and (440) plates of magnetic Fe_3O_4 NPs (Arsalani et al., 2019). The magnetic hysteresis loop of the pure Fe_3O_4 and magnetic chitosan/Fe_3O_4 was measured applying the VSM technique in ± 9 kOe conditions at 298 k. In Fig. 1c, the zero value shown, the coercion and persistence were observed in the curve. The chitosan/Fe_3O_4 sample had a superparamagnetic behavior. The saturated magnetic value was obtained at 44.4 emu/g, which is lower than the Fe_3O_4 magnetic nanoparticles due to the presence of non-magnetic chitosan in the sample structure. It should be noted that the magnetic saturation value for $Fe_3O_4$was 64.3 emu/g. SEM and TEM techniques were used to observe the shape and morphology of magnetic chitosan NPs. SEM image shown in (Fig. 1d) shows the spherical morphology with a particle size of (50−100) nm. In addition,the TEM image (Fig. 1e) to confirm the shape of the NPs, their size was about (10−20) nm. Accumulation of NPs can also be seen.

Analysis of variance (ANOVA) showed significant effects of the investigated factors and their combinations on particular plant yield, quality, and photosynthesis parameters (Supplementary Tables 1 and 2). Significant effects are described in detail below.

3.2. Vegetative yield

With the increasing concentration of foliar sprays of Kitoplus® and Fe-CTs NPs, yield in peppermint plants increased (Fig. 2). The highest fresh weight yield (9132.22 $kg.h^{-1}$) was observed in plants treated with Kitoplus® at a concentration of 0.5% combined with foliar application of Fe-CTs NPs at a concentration of 5 μM (Fig. 2a). The maximum dry matter yield (2308.04 $kg.h^{-1}$) was observed in plants treated with Kitoplus® at a concentration of 0.5% and 10 μM of Fe-CT_S NPs (Fig. 2b).

3.3. Chlorophyll index

In examining the chlorophyll index by SPAD device, it was found that with increasing irrigation and subsequent decrease in stress, the chlorophyll index in peppermint plants increased, and the highest chlorophyll index was observed in plants grown at 60 (51.91) and 30% (52.21) of soil moisture (Fig. 3).

3.4. Stomatal conductance

Results showed that foliar application of Fe-CTs NPs in peppermint plants caused stress and reduced stomatal conductance. The highest stomatal conductance (29.099 cm. S^{-1}) was found in control plants without Fe-CTs NPs treatment (Fig. 4).

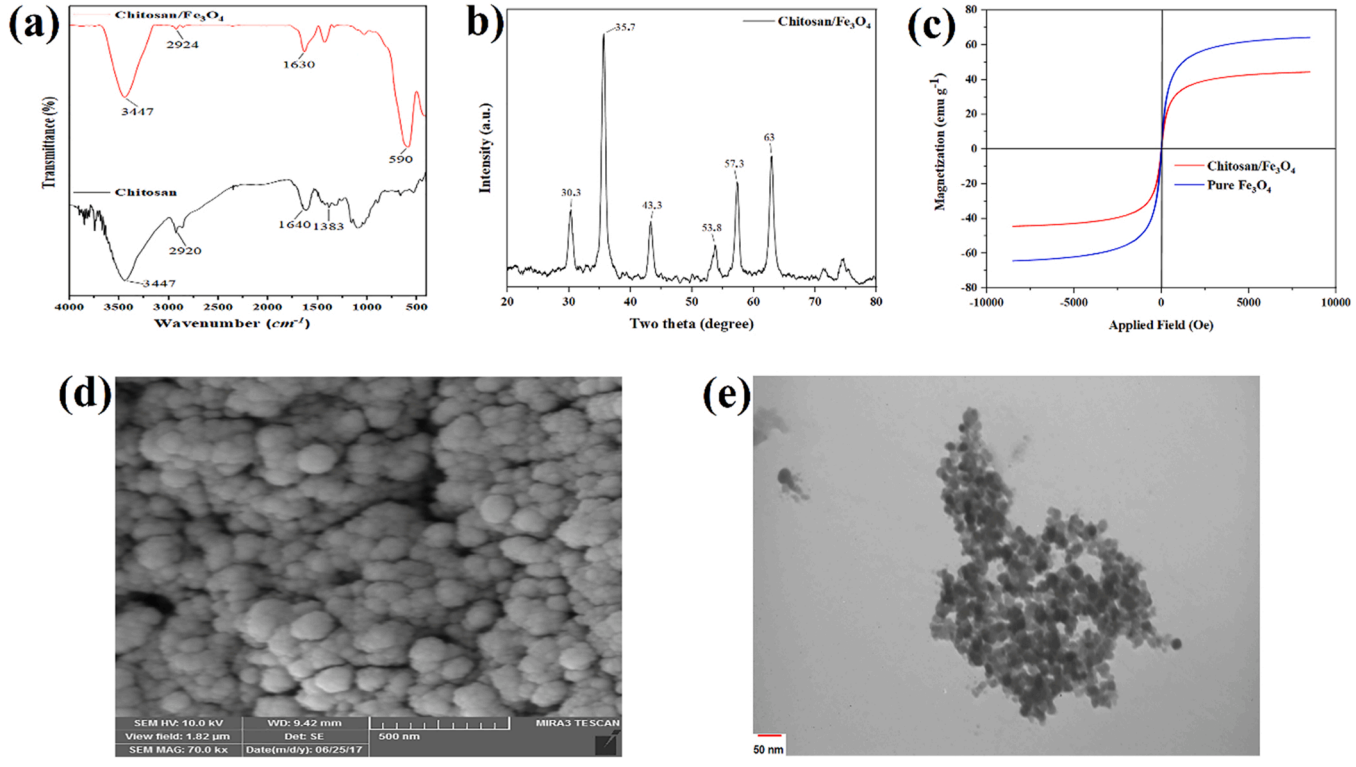

Fig. 1. FT-IR (a), XRD (b), VSM (c), SEM (d), and TEM (f) analyses of starting material and Fe-CT_S NPs.

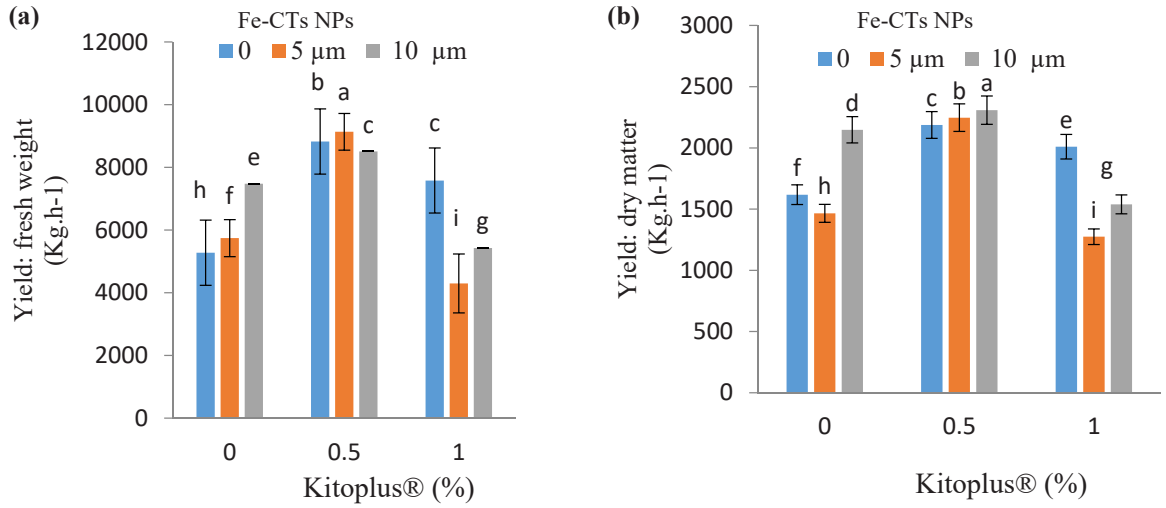

Fig. 2. Effects of Kitoplus® and Fe-CTs NPs on vegetative yield [fresh weight (a) and dry matter (b)] in peppermint plants. (Kitoplus®: 0, 0.5% and 1%. 0, 5, 10 µM Fe-CTs NPs). The values [± standard deviation (SD)] marked with the same letter do not differ significantly according to Duncan's multiple range test ($P < 0.05$).

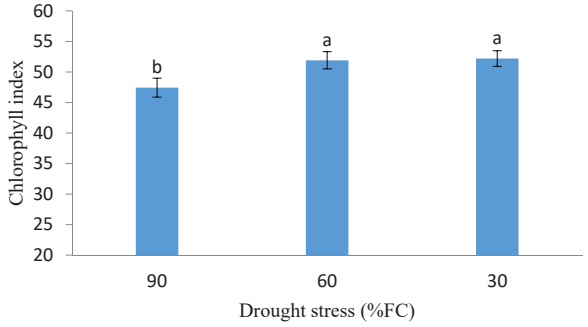

Fig. 3. Relationship between drought stress and chlorophyll index. (90,60%, and 30%: irrigation in three soil moisture levels). The values [± standard deviation (SD)] marked with the same letter do not differ significantly according to Duncan's multiple range test ($P < 0.05$).

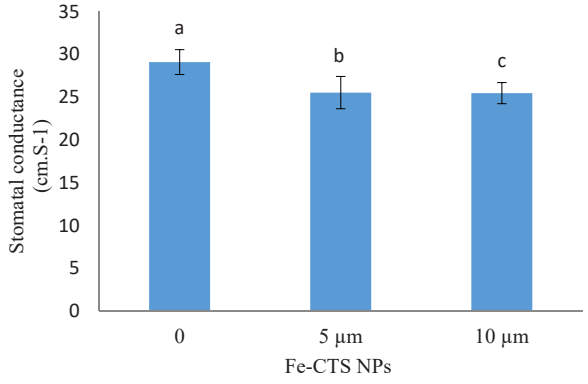

Fig. 4. Stomatal conductance affected by Fe-CTs NPs in peppermint plants. (Fe-CTs NPs 0, 5, 10 µM). The values [± standard deviation (SD)] marked with the same letter do not differ significantly according to Duncan's multiple range test ($P < 0.05$).

3.5. Electrolyte leakage

The increasing drought stress increased the rate of electrolyte leakage in peppermint plants. However, this increase was more pronounced in plants without Kitoplus® treatment, so the highest electrolyte leakage (67.344%) was in control without Kitoplus®, under drought stress at 30% soil moisture. The most significant effect in reducing electrolyte leakage was observed in plants treated with Kitoplus® at a concentration of 1% (Fig. 5).

3.6. Proline

Proline accumulation in plants generally increases with increasing drought stress intensity. In the present study, the highest proline (0.2812 mg.g^{-1}) accumulation was obtained under the interaction of drought stress (30% field capacity) and with foliar applications of Fe-CTs NPs (10 µM) (Fig. 6a). Also, in investigating the effects of drought stress and Kitoplus®, the highest amount of proline (0.2718 mg.g^{-1}) was observed in plants under drought stress at 30% field capacity with Kitoplus® in the concentration of 1% (Fig. 6b).

Along with the increasing concentration of foliar spraying with Kitoplus® and Fe-CTs NPs, the amount of proline accumulation increased so that the highest amount of proline(0.2476 mg.g^{-1}) was observed in peppermint treated with Kitoplus® (1%) and Fe-CTs NPs (10 µM) (Fig. 6c).

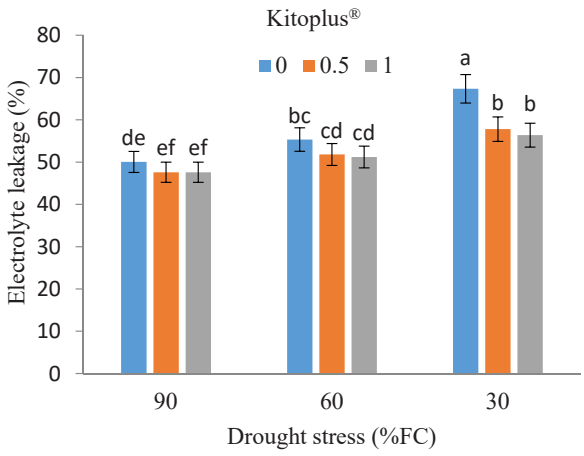

Fig. 5. Interaction of Kitoplus® and drought stress on electrolyte leakage in peppermint plants. (Kitoplus® 0%, 0.5%, and 1%, irrigation in three soil moisture levels (90%, 60%, and 30% of soil field capacity moisture). The values [± standard deviation (SD)] marked with the same letter do not differ significantly according to Duncan's multiple range test ($P < 0.05$).

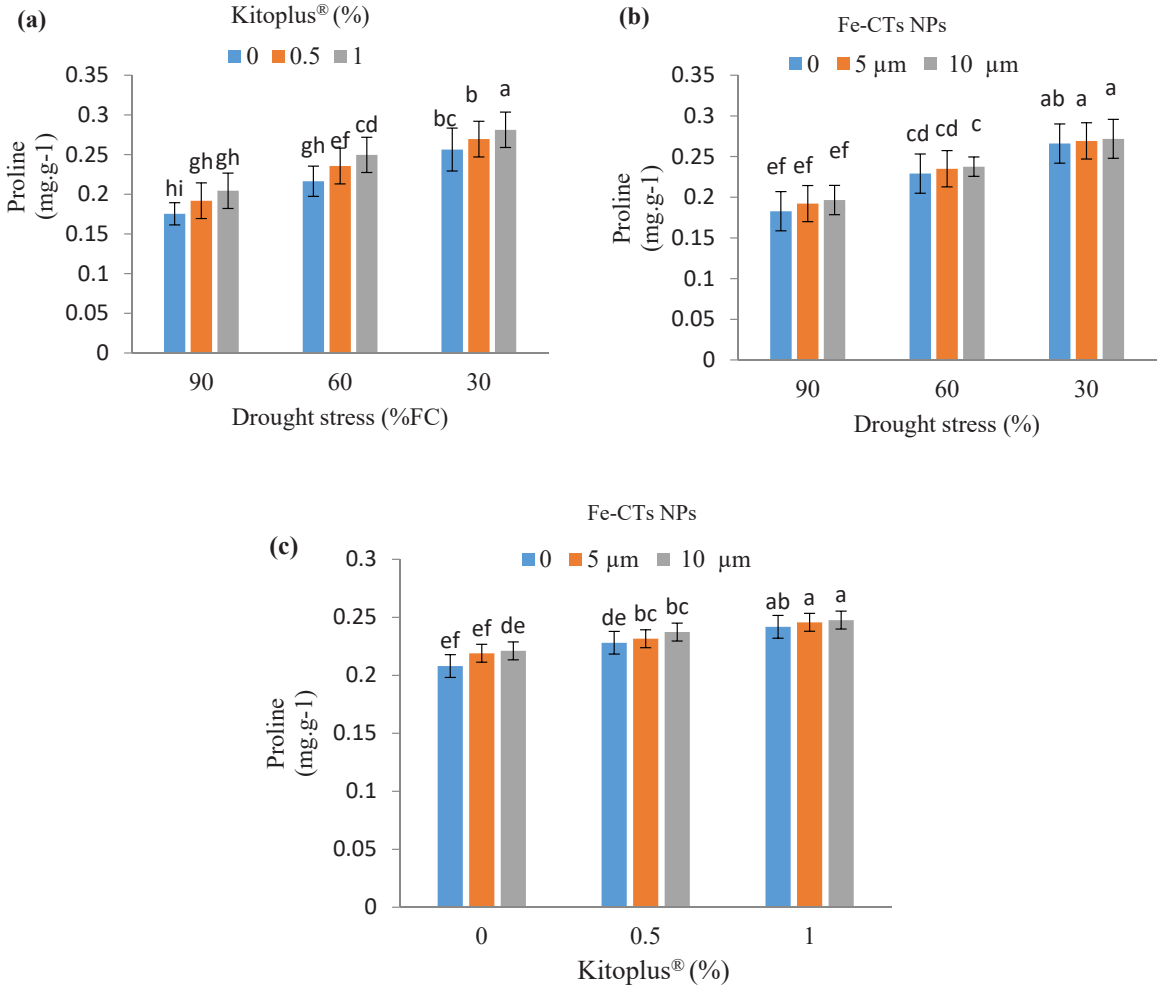

Fig. 6. Proline content affected by Kitoplus®, Fe-CTs NPs, and drought stressin peppermint plants. Kitoplus® at three concentrations (0%, 0.5%, and 1%), irrigation in three soil moisture levels (90%, 60%, and 30% of soil field capacity moisture),Fe-CTs NPs (0, 5, 10 µM). The values [± standard deviation (SD)] marked with the same letter do not differ significantly according to Duncan's multiple range test ($P < 0.05$).

3.7. Essential oils yield and content

In plants under drought stress, with increasing concentration of Kitoplus® essential oil content was increased. The Kitoplus® prevented a sharp decrease in the percentage and yield of essential oils in peppermint plants under drought stress. Moreover, the highest concentration of essential oils (3.488%) was recorded in plants exposed to foliar application of Kitoplus® at a concentration of 1% under soil moisture of 90% (Fig. 7). In general, with increasing drought stress, essential oil yield decreased. The use of Kitoplus® and Fe-CTs NPs in plants without drought stress increased the yield of essential oil (Fig. 8a). The highest essential oil yield (7.9 $g.m^{-2}$) was obtained from plants treated with 1%Kitoplus® under 90% soil moisture (Fig. 8a). In the study of the interactions between Fe-CTs NPs and drought stress, the highest essential oil yield (7.7 $g.m^{-2}$) was observed in plants treated with Fe-CTs NPs at a concentration of 5 µM under irrigation at 90% of soil moisture (Fig. 8b).

The results of GC/MS analysis showed that reducing the amount of irrigation and increasing the concentration of employed treatments caused changes in the percentage of essential oil components (Supplementary fig 2). With increasing drought stress and the concentration of Kitoplus® and Fe-CTs NPs, the amount of β-pinene was increased. Nevertheless, the highest amount of β-pinene was observed in control untreated plants upon drought stress of 30% soil moisture. On the other hand, the amount of β-pinene was reduced by spraying at different concentrations of Kitoplus® and Fe-CTs NPs in water stress of 30% of field capacity due to decomposition or conversion to other essential oil content (Table 3).

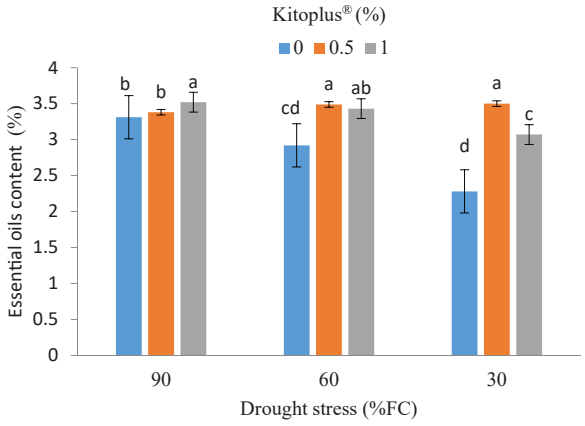

Fig. 7. Effects of Kitoplus® on contents of essential oil (%) in peppermint plants under drought stress. Kitoplus® at concentrations: 0%, 0.5%, and 1%, irrigation in three soil moisture levels (90%, 60%, and 30% of soil field capacity moisture). The values [± standard deviation (SD)] marked with the same letter do not differ significantly according to Duncan's multiple range test ($P < 0.05$).

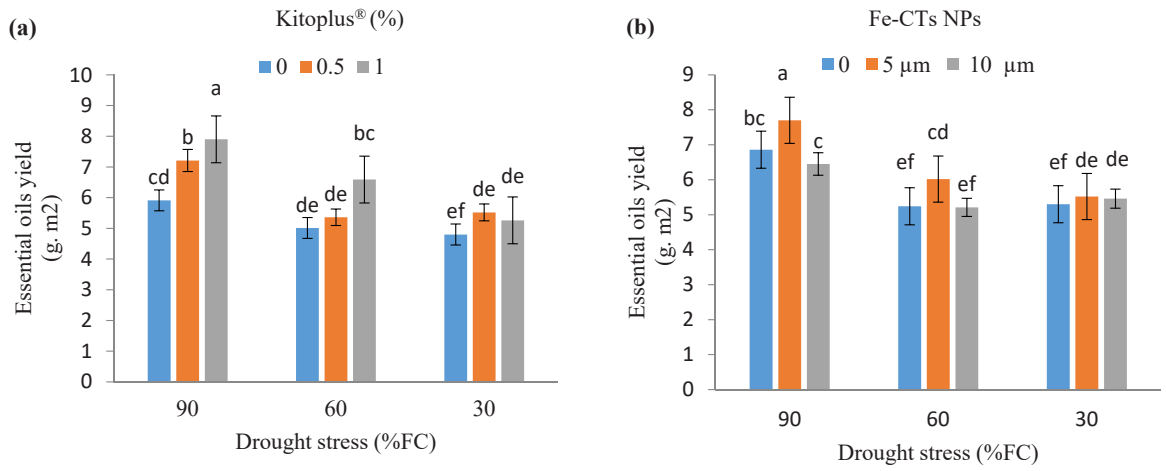

Fig. 8. Essential oil yield changes with Kitoplus® (7a) andFe-CTs NPs (7b) in peppermint plants under drought stress. Kitoplus® at concentrations; 0, 0.5% and 1%, irrigation in three soil moisture levels (90%, 60%, and 30% of soil field capacity moisture),Fe-CTs NPs at concentrations 0, 5, 10 µM. The values [± standard deviation (SD)] marked with the same letter do not differ significantly according to Duncan's multiple range test ($P < 0.05$).

The highest amount of menthol was observed in plants following interaction of Kitoplus® (0.5%) and Fe-CTs NPs (10 µM) under 90% soil moisture. This amount was significantly reduced in plants under 30% and 60% soil moisture. The reduction was more lower in plants treated with Kitoplus® and Fe-CTs NPs than in plants without treatment (control plants) (Table 3). The highest amount of menthone (33.31%) was obtained in plants treated with Kitoplus® at 1% and Fe-CTs NPs at 10 µM under drought stress of 60% soil moisture. It should be noted that the amount of menthone decreased in plants under irrigation stress at 30% of soil moisture (Table 3). The highest amount of L-menthol cyclohexanol, a menthol ester, was observed in peppermint plants treated without Kitoplus® and Fe-CTs NPs at a concentration of 10 µM under drought stress of 60% soil moisture (Table 3). Neomentol levels was higher under irrigation stress conditions of 60% soil moisture, equal to 4.5%, when Fe-CTs NPs were applied at a 5 µM (Table 3).

The amount of menthofuran in treated plants increased with increasing concentration of foliar application of Kitoplus® and Fe-CTs NPs under irrigation stress conditions (from 60% to 30% of soil moisture), and it significantly decreased in plants grown at 90% of soil moisture. This decrease was slighter in plants treated with Kitoplus® and Fe-CTs NPs than that of untreated plants. The highest content of metaforan (23.34%) was observed in plants treated with Kitoplus® at 1% and Fe-CTs NPs at 5 µM under drought stress conditions of 30% soil moisture (Table 3).

3.8. Chlorophyll fluorescence

At moderate drought stress, the Fo in peppermint plants increased. The lowest Fo (248.34) was observed in control plants under irrigation conditions at a moisture content of 90% field capacity (Table 4). In peppermint plants under treatment of Kitoplus® with Fe-CTs NPs, the Fo decreased lower than in control plants grown at the same level of the drought stress. The highest Fo (460.25) was observed in peppermint plants treated with Kitoplus® at concentration of 0.5% along with Fe-CTs NPs at 10 µM under irrigation condition of 60% soil moisture (Table 4).

With increasing drought stress, the amounts of Fm and Fv significantly raised. In line with the Fo, this increase of Fm and Fv in peppermint plants treated with Kitoplus® and Fe-CTs NPs was significantly more prominent than in control plants. The highest Fm (1709.75) and Fv (1358.91) (were observed in peppermint plants treated with Kitoplus® at 1% with Fe-CTs NPs at 10 µM under irrigation stress of 30% soil moisture (Table 4).

The Fv/Fm and the Fv/Fo ratios in drought-stressed plants increased as drought stress raised. The highest Fv/Fm (0.794) and Fv/Fo (3.880) ratios were observed in plants under drought stress (30% of soil moisture) treated with Kitoplus® at 1% and Fe-CTs NPs at 10 µM (Table 4).

4. Discussion

Drought is the most important environmental perturbation cause abnormal molecular, physiological, and metabolism proceedings in plants, adversely restrict plant growth and production. One of the signs of water deficiency is decrease in cellular turgor pressure and, consequently, a decrease in cell growth and development, especially in the stem and leaves. In other words, lowering photosynthetic materials due to the reduction of leaf area and translocation of assimilates to the reproductive organs following drought stress reduces the yield of flowering branches. For this reason, the effect of drought stress on plants can be recognized by the smaller size of the leaves and the lower height of the plants. Leaf development is limited as the leaf area decreases, light absorption reduces, and the total photosynthetic capacity of the plant also decreases (Omid Beigi and Mahmoudi, 2010). Throughout their life cycle, plants deal with various abiotic stress via different physiological pathways. They also alleviate or adapt to diverse stresses by changing gene expression. Experiments have shown that nanoparticles help plants overcome abiotic stresses due to their dependence on plant growth and development (Rahdari and Hoseini, 2012; Mishra et al., 2017).

In the present study, the obtained data showed a decrease in yield of mint plants due to drought stress. However, foliar application of Kitoplus®, especially at a concentration of 0.5%, caused a significant increase in the yield parameters of plants. These results are consistent with the findings of other researchers on using natural growth stimulants (Khan et al., 2002; Gornik et al., 2008). Consistent with the results of this study, Zong et al. (2017) have shown that the use of chitosan in stress conditions in rape (*Brassica rapa*L.) increased plant growth and content of leaf chlorophyll. The chitosan included in Kitoplus® may provide the amino acids, leading to an increase in total N in the leaves or a high ability of plants to absorb N from the soil. Zhang et al. (2017) have shown that the wheat seedlings induced by chitosan could enhance the N reduction and N assimilation. In addition, Kitoplus® may increase the availability, uptake, and transport of essential nutrients by regulating cellular osmotic pressure, thereby improving the number of leaves, shoots, and leaf area by improving plant growth and development, which is effective in improving fresh and dry weight of the plant (Faroukand Ramadan, 2012). Rahman et al. (2018) found that chitosan treatment positively and significantly influenced fresh and dry biomass production in strawberry plants, which is consistent with our findings.

Table 3
Effects of Kitoplus® and Fe-CTs NPs on major essential oil constituents in peppermint plants under drought stress.

A	C (μM)	B (%)	Limonene (%)	Eucalyptol (%)	Menthone (%)	Menthofuran (%)	L-Menthol Cyclohexanol (%)	Menthol (%)	Menthyl acetate (%)	β-Pinene (%)	Pulegone (%)	Caryophyllene (%)
90% of soil moisture	0	0	2.37	5.96	26.75	15.53	25.78	0	0	1.31	3.66	2.47
		0.5	2.19	5.65	25.22	17.5	0	21.6	0	1.2	4.74	2.55
		1	0	6.63	31.5	9.26	0	23.91	0	1.07	3.4	2.12
	5	0	2.44	5.83	29.55	14.8	0	23.58	0	1.39	2.88	4.14
		0.5	2.71	4.8	29.19	15.58	20.41	0	0	1.34	5.78	3.47
		1	2.66	5.84	28.66	15.74	0	25.9	0	1.28	3.44	2.71
	10	0	2.23	5.25	29.89	17.8	21.41	0	0	1.22	4.48	2.32
		0.5	2.58	5.98	31.97	12.72	21.88	0	0	1.52	2.45	2.74
		1	0	5.91	30.43	12.12	0	19.95	0	1.29	5.08	2.62
60% of soil moisture	0	0	0	6.43	28.8	16.16	20.66	3.58	0	1.19	5.13	2.29
		0.5	1.58	6.79	28.77	8.78	24.6	0	0	2.13	3.32	2.78
		1	0	5.93	29.02	13.98	28.09	0	0	1.27	3.73	2.8
	5	0	0	2.06	33.08	3.74	13.49	4.88	0	0.56	7.29	1.23
		0.5	0	7.75	26.98	19.6	1.08	16.66	4.8	1.52	4.53	2.68
		1	0	6.69	26.2	20.62	0	16.03	4.05	1.38	6.3	3.18
	10	0	0	6.63	29.1	23.27	1.21	12.15	5.2	1.48	4.12	3.13
		0.5	0	6.76	26.04	20.01	0	15.81	4.13	0.41	6.55	3.49
		1	0	4.99	33.31	16.86	0	20.51	3.5	1.01	4.36	1.74
30% of soil moisture	0	0	5.83	10.51	4.02	0	0	1.09	1.86	5.1	6.52	1.39
		0.5	0	6.72	27.17	13.11	0	26.5	5.9	0	2.26	2.87
		1	0	7.06	21.18	15.51	0	23.75	4.83	0	4.38	3.39
	5	0	0	5.53	28.84	16.19	0	19.4	4.06	0.88	5.1	3.89
		0.5	3.18	5.4	21.78	16.04	6.62	21.41	6.15	1.07	5.76	3.35
		1	2.56	5.79	23.56	22.77	0	21.44	3.8	1.03	6	2.92
	10	0	2.57	5.61	26.98	17.27	0	23.08	5.31	0.8	4.65	2.92
		0.5	0	5	22.48	23.34	0	21.46	3.89	1.05	6.52	3.39
		1	3.1	4.65	29.2	16.77	0	19.08	4.89	1.05	5.7	3.52

(A: drought stress, B: Kitoplus®, C: Fe-CTs NPs), Kitoplus® at concentrations; 0, 0.5% and 1%, irrigation in three soil moisture levels (90%, 60%, and 30% of soil field capacity moisture), Fe-CTs NPs at concentrations 0, 5, 10 μM.

Table 4
Effects of Kitoplus® and Fe-CTs NPs on chlorophyll fluorescence in peppermint plants under drought stress.

A	C (μM)	B (%)	Fm	Fv	F0	Fv/Fm	Fv/F0
90% of field capacity	0	0	964.03 ± 244.84 kj	653.91 ± 175.36 hi	248.34 ± 25.312 gh	0.524 ± 0.032 hi	1.870 ± 2.25 lm
		0.5	1609 ± 171.51 BCE	1218.75 ± 170.3 de	390.66 ± 54.10 cd	0.755 ± 0.038 BCE	3.172 ± 0.628 gh
		1	1410 ± 529.572 hi	1078.421 ± 60.37 ef	356.50 ± 75.65 de	0.754 ± 0.035 BCE	3.139 ± 0.539 gh
	5	0	1491 ± 296.88 gh	1136.58 ± 284.15 ef	354.41 ± 40.08 de	0.753 ± 0.055 BCE	3.22 ± 0.826 ef
		0.5	1496.58 ± 294.54 gh	1138.253 ± 5.87 ef	358.03 ± 61.62 de	0.756 ± 0.037 BCE	3.183 ± 0.59 fg
		1	1624.66 ± 203.165 BCE	1254.25 ± 195.24 de	370.41 ± 32.60 cd	0.769 ± 0.032 BCE	3.401 ± 0.584 cd
	10	0	1532.213 ± 25.88 ef	1164.58 ± 231.64 cd	367.66 ± 27.44 cd	0.759 ± 0.044 BCE	3.183 ± 0.669 fg
		0.5	1583.143 ± 91.71 de	1224.33 ± 144.5 ef	359.58 ± 19.50 de	0.771 ± 0.024 b	3.410 ± 0.471 cd
		1	1697.119 ± 83.77 a	1350.66 ± 120.34 de	347.16 ± 22.88 de	0.784 ± 0.019 ab	3.901 ± 0.44 a
60% of field capacity	0	0	1291.220 ± 5.7 k	888.199 ± 25.19 gh	403.25 ± 39.42 BCE	0.682 ± 0.041 ef	2.201 ± 0.44 jk
		0.5	1334.20 ± 25.83 k	893.201 ± 66.21 fg	440.58 ± 49.61 b	0.663 ± 0.052 fg	2.047 ± 0.504 kl
		1	1428.206 ± 5.97 hi	993.206 ± 41.03 fg	435.08 ± 45.11 b	0.689 ± 0.048 ef	2.308 ± 0.581 j
	5	0	1384.209 ± 41.81 hi	923.211 ± 91.48 fg	375.50 ± 44.44 cd	0.660 ± 0.055 fg	2.031 ± 0.554 kl
		0.5	1661.81 ± 83.85 b	1245.91 ± 34.79 de	415.91 ± 60.87 BCE	0.721 ± 0.075 cd	2.960 ± 1.62 fg
		1	1611.95 ± 83.92 BCE	1236.58 ± 96.51 de	460.25 ± 28.58 a	0.766 ± 0.021 BCE	3.310 ± 0.396 ef
	10	0	1519.33 ± 59.166 ef	1101.83 ± 95.143 de	417.50 ± 32.01 BCE	0.723 ± 0.019 cd	2.630 ± 0.254 ij
		0.5	1482.167 ± 83.73 gh	1065.41 ± 153.19 ef	417.41 ± 24.81 BCE	0.716 ± 0.026 de	2.550 ± 0.335 j
		1	1505.91 ± 41.18 ef	1090 ± 81.55 ef	415.41 ± 15.90 BCE	0.723 ± 0.012 cd	2.620 ± 0.165 ij
30% of field capacity	0	0	1543.368 ± 66.11 ef	1220 ± 295.9 de	323.66 ± 73.86 ef	0.788 ± 0.014 ab	3.730 ± 0.263 b
		0.5	1631.124 ± 83.37 BCE	1275.33 ± 102.39 cd	356.5 ± 32.76 de	0.781 ± 0.014 b	3.590 ± 0.281 BCE
		1	1535.89 ± 75.66 ef	1192.33 ± 70.84 cd	343.41 ± 21.83 ef	0.776 ± 0.064 b	3.470 ± 0.129 cd
	5	0	1619.1 ± 33.59 BCE	1256.16 ± 88 de	363.16 ± 22.85 de	0.775 ± 0.011 b	3.460 ± 0.231 cd
		0.5	1594.15 ± 66.58 de	1237.41 ± 115.7 de	357.25 ± 42.06 de	0.776 ± 0.012 b	3.470 ± 0.256 cd
		1	1621.77 ± 58.55 BCE	1275.83 ± 62.68 cd	345.75 ± 26.97 de	0.786 ± 0.012 ab	3.701 ± 0.265 b
	10	0	1588.246 ± 75.63 de	1237 ± 89.238 de	351.75 ± 24.91 de	0.771 ± 0.05 b	3.520 ± 0.714 BCE
		0.5	1665.88 ± 41.60 b	1310.66 ± 91.63 b	354.75 ± 23.39 de	0.786 ± 0.017 ab	3.710 ± 0.407 b
		1	1709.75 ± 66.12 a	1358.91 ± 63.58 a	350.83 ± 19.65 de	0.794 ± 0.011 a	3.880 ± 0.296 a

(A: drought stress, B: Kitoplus®, C: Fe-CTs NPs),Kitoplus® at concentrations; 0%, 0.5%, and 1%, irrigation in three soil moisture levels (90%, 60%, and 30% of soil field capacity moisture),Fe-CTs NPs at concentrations 0, 5, 10 μM, the values [± standard deviation (SD)] marked with the same letter do not differ significantly according to Duncan's multiple range tests ($P < 0.05$).

During peppermint's growth and production of active ingredients, a large amount of nutrients is required, and researchers have demonstrated that adequate amounts of micronutrients enhance peppermint's photosynthetic activity. In this research, using Fe-CTs NPs as a foliar application with Kitoplus® caused a significant increase in peppermint's vegetative yield. In agreement with our results, a study of carbon-coated iron magnetic nanoparticles by Gonzalez-Melendi et al. (2008) has shown a significant potential of nanoparticles, in particular, to tackle infections and stress. Using coated nanoparticles as an agrochemical can reduce damage to other plants and decrease excessive chemical emissions to the environment. Harsiniet al. (2014) have shown that foliar application of iron nanoparticles chelates in wheat had significant effects at the 5% probability level on harvest index, biological yield, and grain yield (Harsini al, 2014). Because it is produced with inexpensive raw materials such as chitosan, Kitoplus® is much more cost-effective than similar fertilizers. By examining the cost of growing mint on farms, it turned out that the method used in this experiment was more cost-effective than similar approaches used by farmers.

Our result showed that the stomatal conductance decreased with the increased concentration of Fe-CTs NPs. It seems that in peppermint, after applying Fe-CTs NPs, the plant has limited the evaporation of leaf water by closing the leaf stomata.

In the current study, proline content increased in drought-stressed plants. Stress generally causes a higher accumulation of proline and osmolytes in plants. The accumulation of osmolytes such as soluble sugars, amino acids, and other compatible solutes is a typical plant response to water stress (Sharma et al., 2019), and the use of Kitoplus® with Fe-CTs NPs increased proline accumulation in peppermint plant. In agreement with these results, Emami et al. (2017) showed that the compensatory effect of chitosan in reducing the negative impact of stress conditions on the dry matter was due mainly to stimulation of osmotic adjustment through proline accumulation in *Thymus daenensis* Cela.

A comparison of the content of essential oils in peppermint plants treated with Fe-CTs NPs and Kitoplus® under drought stress shows differences in the type of compounds. Compounds such as menthol, menthone, germacrene-D, and terpenoids such as pinene have been identified in most members of the mint family (Schmidt al, 2009; Kumar et al., 2004). The environmental conditions and type of nutrition result in many differences in the percentage of these substances in peppermint essential oil. In this study, the highest oil content of peppermint was found in plants growing under mild drought stress (under irrigation conditions of 60% soil moisture). In agreement with our study, Jahani et al. (2021) showed that the lowest oil content was from plants under regular irrigation regimes. The most crucial parameter of peppermint quality is the amount of menthol aromatic substances in its essential oil, and its economic and medicinal value depends on the harvested amount of menthol (Smeti al, 2013). According to our results, the increase of peppermint essential oil compounds such as menthol and menthone was probably due to more readily accessible micronutrients (Fe-CTs NPs). As a result of using the appropriate combination of the Fe-CTs NPs and Kitoplus®, the canopy volume and the plant's leaf area increased. In agreement with our study, Ahmad et al. (2019) have shown that the application of chitosan increased menthol and menthone contents of *M. piperita* essential oil. In other studies, Goudarzianet al. (2021) have found that the foliar-applied chitosan andinoculation with arbuscular mycorrhizal fungi improved the quantity and quality parameters of active substances of peppermint, such as the contents of essential oil, menthol content, and balance of menthol/menthone.

Chlorophyll fluorescence parameters have been extensively used to evaluate the effects of drought stress on plant photosynthetic systems and to estimate the efficiency of photosystem II (QII). When stress occurs, the energy distribution changes between the fluorescence, photochemical, and non-photochemical or heat dissipation components. Any factor that reduces photosynthesis or is stressful for the system increases the fluorescence of chlorophyll. The amount of chlorophyll fluorescence indicates the integrity of the thylakoid membrane and the relative efficiency of electron transfer from photosystem (QII) II to photosystem I (Rombol et al., 2005). In particular, the response of plants varies and depends on the severity of the stress and the recovery time. Oh-Kerry and Rajashkar (2019) have shown that regulated water deficiency

positively affects phytochemical concentrations in plants without any adverse effects on growth. However, the response to different stresses is dependent on the magnitude and type of stress and plant genotype (Shin et al., 2021). Our study showed the differential effect of drought stress on chlorophyll fluorescence parameters, which was dependent on the stress and treatment conditions.

In the present study, the amount of Fv increased at all levels of drought stress. The increase was more significant in control plants compared to those without treatments. This increase can be due to the inhibition of the prevention of electron transfer from the donor side of photosystem II to the site of electron reception by quinine molecules (QC and QA) and inhibition of photosystem II photosynthesis (Gebeyehu et al., 2010). In many studies examining the effect of stress on plants, the Fv/Fm index has been used. The Fv/Fm ratio indicates that the maximum quantum efficiency of photosystem II is when all the reaction centers of photosystem II are open.In many plant species, Fv/Fm ratios of 0.83 and above indicate that the plant is not under stress (Maxwell and Johnson, 2000). The Fv/Fm is evaluated as an effective tool in detecting damage to the photosynthetic apparatus before it is detected in plant morphology.

In the present study, control plants under severe stress had a more significant decrease in Fm, so with increasing drought stress, control plants had less photosynthetic efficiency compared to plants under foliar sprays with Kitoplas® and Fe-CTs NPs. On the other hand, decreased Fv/Fm indicates lower efficiency of photosystem II. This ratio has decreased due to an increase in Fo, a decrease in Fm, or both. Water restriction appears to reduce the photochemical efficiency of photosystem II due to an increase in Fo. The decrease in Fv/Fm in this experiment in control plants is probably due to damage to chloroplasts, and the decrease in chlorophyll content in control plants under drought stress confirms this. Although there have been few studies on the effect of natural growth stimulants and nanoparticles on chlorophyll fluorescence parameters, in the case of Kitoplus®, which is used as a biofertilizer and growth stimulant, it has been shown that in plants under salinity stress and drought, it can increase the quantum efficiency of photosystem II. In this regard, we can refer to studies on rice (Ruiz-Sanchez et al., 2011) and corn (Zhu et al., 2012) under drought stress.

The proposed mechanism for the nanomaterials-induced mitigation of drought stress adverse effects in plants based on the results of the present study and related literature is given in Fig. 9. Drought stress induces remarkable variations in different physiological, biochemical, and molecular processes, depending on the period of stress exposure, extent and severity of the stress, cell specific mechanisms, types of genotypes, and developmental stages which ultimately decrease biomass accumulation and yield in several agricultural crop plants (Baiazidi-Aghdam et al., 2016; Wang et al., 2018; Iqbal et al., 2022). The negative effects of drought stress are mainly due to the overproduction of reactive oxygen species (ROS) and the inhibition of photosynthesis, which subsequently affect other physiological processes (Cruz de Carvalho, 2008). However, drought stress increase the actual biosynthesis rate of essential oils in plants cells due to a passive shift in their redox status. In addition, the activity of enzymes involved in biosynthetic pathway of the essential oils (e.g., phenylalanine amonia-lyase, PAL) found to be up-regulated following exposure to various types of biotic and or abiotic stresses (Selmar et al., 2017). Thus, the economical yield of medicinal plants may be notably improved by applying methodically drought stress during their cultivation. Several mechanisms underlying the nanomaterial-mediated mitigation of drought stress can be anticipated based on the present study and related literatures such as (i) upregulation of enzymatic and non-enzymatic antioxidants, which

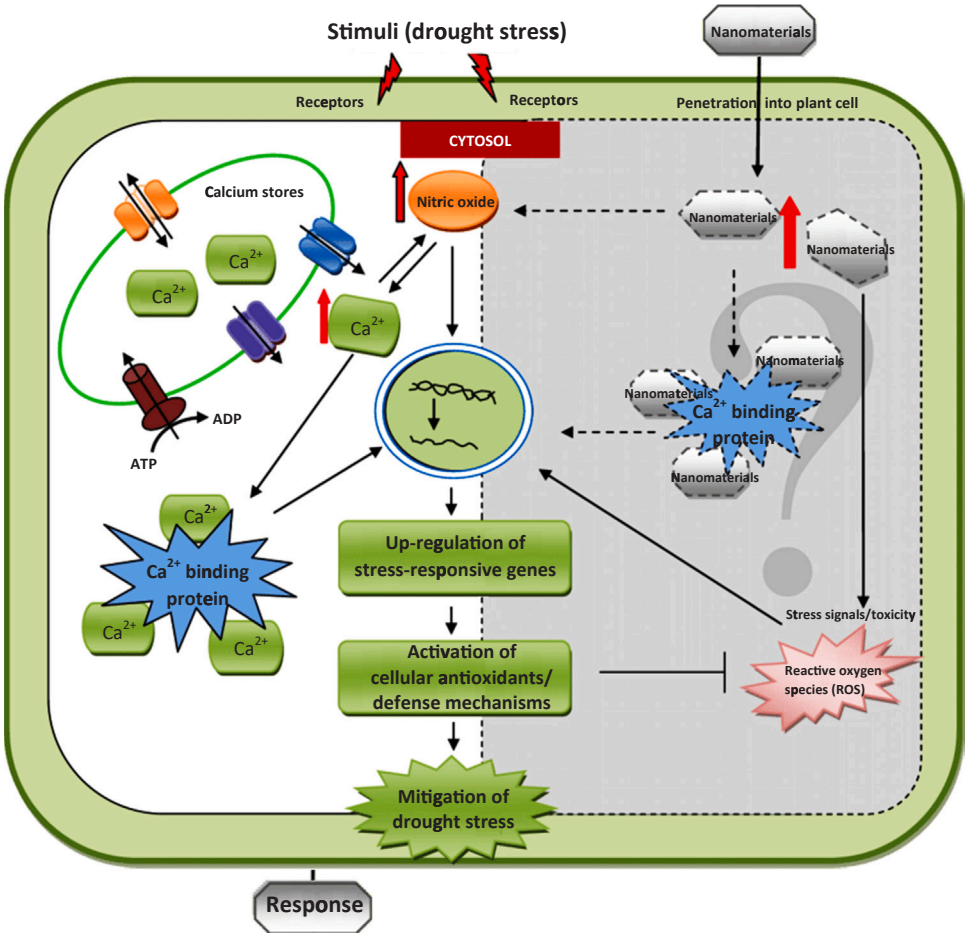

Fig. 9. Plants perceive stress stimuli via receptors located on plasma membrane. Perception of abiotic stress stimulus like the drought stress elevates the cytosolic Ca^{2+} level in plant cells. Increased intracelluar Ca^{2+} concentration is sensed by Ca^{2+} binding protein. Ca^{2+} binding/signaling consequently regulates gene-specific transcription factors, which either promotes or inhibits their expression machinery causing to plant tolerance to the stress (Ghosh et al., 2022). Exposure to nanomaterials led to over-expression of Ca^{2+} binding proteins, and bind to Ca^{2+} binding protein trigger downstream signaling events/pathways, and ultimately the expression of stress associated genes and activation of plant's enzymatic and non-enzymatic reactions against stress. Nanomaterials also induce nitric oxide biosynthesis, which is belived to act as an antioxidant molecule, scavenging reactive oxygen species (ROS) during oxidative stress and diminishing lipid peroxidation (Chandra et al., 2015). Nitric oxide also mediates photosynthesis process and stomatal conductance and regulates programmed cell death, therefore, providing tolerance to drought stress. Application of appropriate concentrations of specific nanomaterials, by regulating antioxidative defense mechanisms, also maintains ROS at the basal level sufficient enough for normal cell functioning and homeostasis as well as stress signaling leading to activation of plant's defense system and stress tolerance.
A schematic representation of cellular defense mechanism in drought stress conditions and under the influence of nanomaterials (adopted and modified from Khan et al., 2017).

directly play as ROS scavenger, and or serve as signal to produce constituents of the antioxidant system, (ii) the improvement of essential oils production, which regulate physiological processes under drought stress (iii) osmoregulation via the maintenance of organic compounds such as proline, and the retention of water in the leaf tissue (Hatami et al., 2017; Chegini et al., 2017; Ghorbanpour et al., 2020). Additional details are illustrated in the Fig. 9.

5. Conclusions

In this study, foliar application of growth stimulants of Kitoplus® and Fe-CTs NPs affected the performance of peppermint, especially upon drought stress conditions, and alleviates the negative effects of drought stress to some extent. Kitoplus®' along with Fe-CTs NPs increased the chlorophyll fluorescence of plants by reducing irrigation stress at 60% soil moisture. Plants treated with Kitoplus®at a concentration of 0.5% produced a higher yield than that of control plants. In the study of chlorophyll fluorescence under drought stress, however, plants treated with natural growth stimulant along with Fe-CTs NPs, showed more significant increase in yield than that of control plants. With decreasing irrigation rate and increasing the concentration of treatments used, essential oil components were directly realted to change. Plants exposed to drought stress of 90% field moisture were significantly affected by interactions between Kitoplus® at 5% and Fe-CTs NPs at 10 μM. Thus, it seems Kitoplus® and Fe-CTs NPs can be used instead of chemical fertilizers to increase the percentage of essential oils and their major constituents in medicinal plants and achieve sustainable agricultural goals. In general, 60% drought stress level with a concentration of 10 μM of Fe-CTs NPs along with Kitoplus® (at a concentration of 0.5% and 1%) were very useful on the value of major essential oil compounds in peppermint such as menthon and menthol, which are the most valuable ingredients in peppermint oils. Therefore, the use of these concentrations is recommended in subsequent experiments. However, further researches at sub-cellular and molecular levels are needed to be ensure whether different types of nanomaterials act as stress mitigators and/or stress inducers in several plant species.

CRediT authorship contribution statement

Mousa Torabi Giglou: Writing – original draft, Writing – review & editing, Supervision, Project administration, **Rasoul Heydarnajad Giglou:** Writing – original draft, Writing – review & editing, Conceptualization, Methodology, Software, Visualization. **Behrouz Esmaeilpour:** Writing – original draft, Writing – review & editing, Conceptualization, Methodology, Software. **Rasoul Azarmi:** Writing – original draft, Writing – review & editing, Conceptualization, Methodology, Software. **Jadwiga Śliwka:** Writing – original draft, Writing – review & editing. **Gholamreza Gohari:** Writing – original draft, Writing – review & editing, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2022.115286.

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