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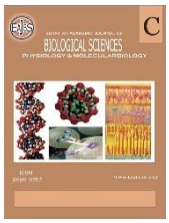
Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 16(1) pp75-92 (2024)*
DOI: 10.21608/EAJBSC.2024.336730

Egypt. Acad. J. Biolog. Sci., 16(1):75-92 (2024)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Impact of Titanium Dioxide Nanoparticles on the Evaluation of Mint Oil (*Mentha Spicata* L.) Using some Physiological Analyses and Molecular Markers

Salma Metwally; Amr H. Nassar and Hala El-Atroush
Botany Department, Faculty of Science, Ain Shams University
***E-mail:** Salma_metwally@hotmail.com

ARTICLE INFO
Article History
Received:21/11/2023
Accepted:16/1/2024
Available:20/1/2024

Keywords:
Mentha spicata L., Titanium dioxide nanoparticles, Molecular Markers, Mint oil.

ABSTRACT

To evaluate the impact of Titanium dioxide nanoparticles on pigments, minerals, and essential oil of *Mentha spicata* L. five treatments of biologically and chemically synthesized TiO_2NPs were applied to spearmint leaves. Results showed that 50 mg/L BNT-treated plants had the highest contents of chlorophyll (a) and total chlorophyll, while 25 mg/l CNT-treated plants recorded the highest carotenoids and micronutrients (Fe, Mn, Cu, Na, and Zn) contents. Spearmint oil constituents were determined by Gas Chromatography-Mass spectroscopy (GC-MS), which demonstrated the positive effect of TiO_2NPs on improving the spearmint oil constituents, as some therapeutic compounds appeared in CNT, BNT, CNTN, and PC compared to the negative control. Also, the percentage of some compounds increased in all treatments, while the percentage of the toxic compound isopulegone decreased or disappeared at all.

Random Amplified Polymorphic DNA (RAPD) markers were also used for the assessment of genetic variation between different treatments of Titanium dioxide nanoparticles on *Mentha spicata* L. in comparison with the control. A total of 10 arbitrary sequence primers were evaluated. All 10 primers used for the RAPD analysis showed consistent band patterns. In total, scorable bands were observed with the primers. The total number of amplicons produced per primer varied from 7 for OPA-09 and OPB-10 to as many as 13 bands for OPA-4. The average number of bands per primer was 9.9. Out of 99 bands, 31 were polymorphic (31.31%). The average number of polymorphic RAPD bands was 3.1 per primer. The highest similarity (94%) with the negative control was recorded in spearmint leaf treated with Titanium dioxide nanoparticles that were chemical source-derived amended with nitrogen, while spearmint leaf treated with Titanium dioxide nanoparticles that biological source-derived amended with nitrogen was found to show the least similarity (88%) with the negative control. In this concern, RAPD results have been suggested to be useful fast methods for comparing genetic changes and variation in plants.

Overall, the results of the present investigation indicated that the foliar application of TiO_2NPs (CNT, BNT, and CNTN) on *Mentha spicata* L. has a significant positive effect on micronutrients and pigment contents, besides improving the spearmint oil quality. The differences within treatments were also affirmed by the genetic markers of the RAPD technique.

INTRODUCTION

The development of the agriculture system of any country is a point of concern to suit its needs (Shafi et al., 2020). Nanotechnology is a novel scientific technique that combines the use of materials and equipment capable of exploiting the physical and chemical features of a substance at physiological and molecular levels to investigate numerous themes ranging from medicine to agriculture. It is the science and technology of tiny materials less than 100 nm in size (one nanometer is 10^{-9} meters) (Fakruddin *et al.,* 2012).

Nanotechnology is an emerging technology that has the potential to significantly assist agriculture, potentially resulting in a new revolution. According to Shabir *et al.,* (2019), TiO_2NPs have piqued the interest of scientists due to their plant growth elicitor activity. Furthermore, TiO_2NPs would benefit current agricultural farming by increasing the efficiency of nutrient uptake (Kleiber and Markiewicz, 2013). TiO_2NPs also increased the plant growth profile (shoot and root length, leaf area) and biochemical attributes for antioxidant enzymes, chlorophyll, carbohydrate, and protein contents (Sadak, 2019).

Mint is one of those medicinal plants that are being used worldwide. It has great importance in folk medicine and the food industry. The genus Mentha belongs to the family Lamiaceae, consisting of about 25 to 30 species. Classification of the genus *Mentha* is complicated due to the elevated incidence of polyploidy, a wide range in the morphology and numeral variation in chromosomes, recurrent inter-specific hybridization, and vegetative spread. Natural inter-specific hybridization occurs with high frequency both in cultivated and wild populations of *Mentha*. Several cytological, morphological, chemical, and molecular markers have been reported to reveal relationships among Mentha species (Ibrahim, 2017).The most common species of the genus *Mentha* are *M. spicata, M. piperita,* and *M. aquatica* (Tucker, 2007). *M.spicata* (spearmint) has volatile oils that are economically important for their food flavoring characteristics (Ay Kee, *et al.,* 2017). It`s clear that the interaction between nanoparticles and plants is very complicated and depends on many factors, such as the shape, size, crystallinity, and dosage of nanoparticles. Additionally, nanoparticles can affect the genetics of plants (Tripathi *et al.,* 2017). Moreover, the application of nanoparticles to plants can currently enhance traits or induce the appearance of new ones. In this concern, molecular markers such as RAPD, ISSR, and SRAP have been successfully indicating the changes in DNA fingerprints and determining the genetic variations (Siddiqui *et al.,* 2015).

The development of the Polymerase Chain Reaction (PCR) technique has revolutionized the field of molecular biology (Coleman and Tsongalis, 2005). The DNA fingerprinting technique of Randomly Amplified Polymorphic DNA (RAPD) provides an unlimited number of markers that can be used for various purposes (Sabri and Saeed, 2023). RAPD markers can be generated using short arbitrary primers to amplify genomic DNA, giving a genotype-specific pattern of bands. RAPD markers are the most widely used molecular technique for DNA fingerprinting. The RAPD technique has become an increasingly popular tool in genetic studies (Emadpour *et al.,* 2009).

Molecular markers such as RAPD have been suggested to be useful for the confirmation of genetic fidelity in plants (Soliman *et al.,* 2018). This technique has the advantages of giving reproducible results, low cost, and designing primers without any prior knowledge of sequences (Kumari and Thakur, 2014). RAPD is a simple, dominant, quick, and easy assay marker that requires low quantities (5-50 ng) of template DNA (Wahyudi *et al.,* 2020). RAPD markers reveal a differential effect of Titanium dioxide nanoparticle treatments on the genomic DNA of spearmint plants that help identify more (or less) genetic variation.

The present study focused on the applications of nanotechnology in agriculture sector improvement and plant nutrition by estimating the physiological and genetic variations among spearmint plants and evaluating the impact of different treatments of Titanium dioxide nanoparticles on *Mentha spicata* L. by using some physiological analysis and the Random Amplified Polymorphic DNA (RAPD) technique. Also, detects the viability and efficiency of the RAPD marker and the genetic variation between different treatments.

MATERIALS AND METHODS

Chemicals and Reagents:

All chemicals were of analytical grade and purchased from Merck, Germany, except concentrated sulfuric acid, which was from Fluka, 98%. Double-distilled water was used throughout this investigation.

Plant Material:

Mentha Spicata L. was propagated by means of healthy rhizomes which were obtained from Mazhar Botanical Garden (MBG), 26th of July corridor, Barageel district, Nahia, Imbaba, Giza Governorate, 12511 Egypt. All rhizomes were gathered in the spring of 2018 and were cultivated under controlled conditions inside the greenhouse at the Faculty of Science, Ain Shams University, Cairo, Egypt. Then when seedling stems were getting 2-3 true leaves, they were transferred into 15 cm pots (clay, washed sand, and peat moss (1:2:1) with pH 6.5 (± 0.2). All the agricultural practices were done whenever necessary. For the first three weeks, pots were irrigated with a quarter-strength modified Hoagland solution with pH 5.5-6.0 (± 0.2) and then with half strength until the end of the experiment. The pots were irrigated with pure water once a week to wash away salt accumulation (Bussler, W. 1972).

Green Net House Experiment:

To study the effect of Titanium dioxide nanoparticles on mint oil, this experiment was designed.48 pots of *Mentha Spicata* (Spearmint) plants were used for nanoparticle experiments; by dividing them into 6 groups (8 pots per group), each one has a specific treatment. All TiO_2NPs treatments were applied after a month of cultivation by foliar spray with concentrations of 25 mg/l and 50 mg/l for chemically and biologically synthesized Titanium dioxide nanoparticles respectively. (1): pots treated with chemically synthesized TiO_2NPs (CNT) at a concentration of 25 mg/l and were irrigated with half-strength modified Hoagland solution only. (2): pots treated with biologically synthesized TiO_2NPs (BNT) at a concentration of 50 mg/l and were irrigated with half-strength modified Hoagland solution only. (3): pots as a negative control (without any treatment) (NC) and were irrigated with half-strength modified Hoagland solution only. (4): pots treated with chemical TiO_2NPs and were irrigated with half-strength modified Hoagland nutrient solution amended with potassium nitrate with a concentration of (4.5ml/l) as a nitrogen source (CNTN). (5): pots treated with biological TiO_2NPs and were irrigated with half-strength modified Hoagland nutrient solution amended with potassium nitrate with a concentration of (4.5ml/l) as a nitrogen source (BNTN). (6): pots as a positive control (PC) (irrigated with half-strength modified Hoagland nutrient solution amended with potassium nitrate with a concentration of (4.5ml/l) without TiO_2NPs.

Sources of Nanoparticles:

Titanium dioxide nanoparticles were obtained by 2 methods as follows:

Chemical Method: Titanium dioxide nanoparticles in anatase form were purchased from the Nanotechnology Research Center, Faculty of Engineering, Minufiya University.

Biological Method: Titanium dioxide nanoparticles were synthesized at the Faculty of Science, Ain Shams University, Cairo, Egypt.

Several trials were carried out to determine the most effective nitrogen source to be used in media preparation to obtain the most suitable, effective size, and appropriate amount of biological anatase TiO_2-NPs.

1-By using different nitrogen sources (peptone, ammonium nitrate, and potassium nitrate) were separately in the preparation method of yeast growth media.

2- Changing the ratio of sucrose as a sugar (disaccharides) carbon source (one time 1 g is used and another time 2 g).

For each nitrogen source with one of the two concentrations of sucrose per 100 ml of the nutrient medium, 8 replicates were used and inoculated with the same amount (volume) of yeast and incubated at 25°C for 36 hours. After that, the formation of the biological anatase TiO_2-NPs will be performed and a comparison between the amount and size gained from each one was detected.

As a result of the previously mentioned trial preparations, we determined that the highly significant growth medium to be used in our study was a ratio of 2:2:1 mixture of peptone (as a nitrogen source), sucrose, and yeast extract respectively.

Preparation Method of Biological Anatase Titanium Dioxide Nanoparticles using Yeast (Saccharomyces service):

Biological anatase Titanium dioxide nanoparticles (B-TiO_2NPs) were prepared according to the modified method of Jha *et al.*, (2009).

Initially, two separate sets of media were prepared (100 ml will be used for yeast inoculation and another 300 ml will be used for medium dilution). The medium was prepared by adding 2 g peptone, 2 g sucrose, and 1 g yeast extract to 100 ml of distilled water (with a ratio of 2:2:1), then it was sterilized by autoclaving. Following that, an amount of approximately 10 g of yeast (*Saccharomyces cerevisiae*) was added to the 100 ml of medium and gently shaken until heavy bubbles appeared on the medium surface, after that, yeast cells were allowed to grow as a suspension culture for approximately 36 hours in an incubator, and this was treated as a source culture.

The media with the growing yeast was filtered by Whatman (No.1) filter paper, and was calibrated and diluted four times by adding 30% ethanol-containing nutrients, and was incubated at room temperature for approximately 24 hours until it attained a straw yellow color, then for each 100 ml of media, 20 ml of 0.025 M metatitanic acid were added, and it was heated in a steam bath up to 60°C for 20 minutes till the appearance of white depositions, then it was allowed to be cooled and incubated at room temperature for 12-48 hours. Finally, the suspension was centrifuged at 20°C for 20 min at 4800 rpm to obtain the biologically synthesized Titanium dioxide nanoparticles.

Characterization of Nano Titanium Dioxide Particle Size:

The particle size of n-TiO_2 was measured by XRD (x-ray diffraction) at the Metallurgical Development Research Center, Al Tbin, Cairo.

Physiological Analyses:

Photosynthetic Pigments In Spearmint:

Extraction and Determination Of Photosynthetic Pigments (chlorophyll a, chlorophyll b and carotenoids):

The extraction and determination of chlorophyll a, chlorophyll b, and carotenoids content from the healthy and fully expanded mature leaves were carried out using the methods described by Sumanta *et al.* (2014) and E. Straumite et.al. (2015), respectively. The equations that were used in pigment calculations are demonstrated in Table (1).

Mineral Contents:

Metal Determination Method by ICP-MS:

For metals determination, harvested plant materials were dried at 65 ◦C for 72 h and then ground with a mill. A total of 0.5 g of each sample was then digested with 10 ml of a mixture of 69% HNO_3, conc HCl (3:1v/v) in a heating digester (DK 20, VELP Scientifica, Milan, Italy).

Plant extracts were filtered through disposable 0.2 µm PTFE syringe filters (DISMIC-25HP, Advantec, Tokyo, Japan). The metal concentrations in these extracts were determined by means of inductively coupled plasma-mass spectroscopy (ICP-MS) (iCAP, Thermo, Germany) at the central lab of Genetic Engineering and Biotechnology Research Institute (GEBRI). Certified reference materials (Merck, Germany) were included in the analyses. The recovery of metals was within the certified limits. Qtegra software was used for average and relative standard deviation calculation.

Table 1: The equations for the calculation of chlorophyll a, chlorophyll b, total chlorophyll, the Ratio between chlorophyll a and b, and the total carotenoids content (Sumanta *et al.*, 2014). Units are presented as a mg ml^{-1}

Parameters	*Equations
Chlorophyll a	C_{Cha} =12.25 A662 -2.79 A645
Chlorophyll b	C_{Chb} = 21.5 A645 -5.1 A662
Total chlorophyll	$C_{Cht} = C_{Cha} + C_{Chb}$
Ratio between chlorophyll a and b	Ra/b = C_{Cha} / C_{Chb}
Total carotenoids	C_{ca}=1000A470-1.82C_{Cha}-85.02C_{Chb}/ 198

In column Equations, the following abbreviations are used: C_{Cha} – concentration of chlorophyll a; C_{Chb} – concentration of chlorophyll b; C_{Cht} – concentration of total chlorophyll; Cca – concentration of carotenoids; A662 –absorbance of at wavelength 662 nm; A645 – absorbance at wavelength 645 nm; A470 – absorbance at wavelength 470 nm.

Spearmint Oil Composition:

Extraction and Determination Method of Mint Oil Constituents:

Pure Mint oil which was extracted from the dry leaves of the mint plant by hydro-distillation, was used for the determination of mint oil constituents. In this concern,

Chromatographic analysis using GC–MS was performed (Agilent Technologies 7890B GC Systems combined with 5977A Mass Selective Detector). A capillary column was used (HP-5MS Capillary; 30.0 m × 0.25 mm ID × 0.25 μm film) and the carrier gas was helium at a rate of flow of 1.8 ml/min with 1 μl injection. The sample was analyzed with the column held initially for 3 min at 40 °C after injection, then the temperature was increased to 300 °C with a 20 °C/min heating ramp, with a 3.0 min hold. Injection was carried out in split mode (10:1) at 300 °C. MS scan range was (*m*/*z*): 30–550 atomic mass units (AMU) under electron impact (EI) ionization (70 eV). The volatile oil constituents were determined by mass fragmentations with The NIST mass spectral search program for the NIST/EPA/NIH mass spectral library Version 2.2 (Jun 2014), and data were expressed as percentages (%).

Statistical Analysis:

The experiments were set up in a completely randomized design. Data were statistically analyzed by analysis of variance (ANOVA) using Co-Stat (Version 6.311) software program to detect significant differences among the mean of treatments using Duncan's multiple range test (DMRT) at a 5% significance level.

Molecular Analyses:

Evaluation of Polymorphism in Different Treatments Using Molecular Markers Plant Materials and DNA Extraction

Fresh samples (group 1 to group VI) were frozen in liquid nitrogen and stored at -80°C. Total genomic DNA was isolated from the six group samples, (CNT, BNT, NC, CNTN, BNTN, and PC) as mentioned prior that were chosen previously, All samples were analyzed by Random Amplified Polymorphic DNA (RAPD) using ten oligonucleotide primers; OPA-01, OPA-03, OPA-04, OPA-05, OPA-09, OPA-12, OPB-02, OPB-05, OPB-08 and OPB-10 (Table, 2) according to Kabir et al., (2014).

DNA Extraction and Purification:

DNA extraction was carried out by a modified CTAB (hexadecyl trimethyl ammonium bromide) method according to Porebski *et al*. (1997), CTAB buffer was used within 2-3 days, store capped: add polyvinyl pyrrolidone molecular weight 40,000 (PVP-40) and β-mercaptoethanol and stir to dissolve before starting extractions. The DNA quantity was estimated using Nanodrop device at 260 nm. The final DNA concentrations were adjusted and stored at -20°C.

Table 2: List of RAPD primers, their names, nucleotide Sequences, and GC % used for present analysis.

Primer	Sequence	GC %
OPA-01	CAGGCCCTTC	70
OPA-03	AGTCAGCCAC	60
OPA-04	AATCGGGCTC	60
OPA-05	AGGGGCTTTG	60
OPA-09	GGGTAACGCC	70
OPA-12	TCGGCGATAG	60
OPB-02	TGATCCCTGG	60
OPB-05	TGCGCCCTTC	70
OPB-08	GTCCACACGG	70
OPB-10	CTGCTGGGAC	70

Random Amplified Polymorphic DNA (RAPD) Analysis:

The amplification reaction was carried out in 25 µl reaction volume containing 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 25 pmol primer, 1 U *Taq* DNA polymerase and 30 ng template DNA.

PCR amplification was performed in a PerkinElmer/Gene Amp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 35 cycles after an initial denaturation cycle for 5 min at 94°C. Each cycle consisted of a denaturation step at 94ºC for 45s, an annealing step at 36ºC for 50s, and an elongation step at 72ºC for 1 min. The primer extension segment was extended to 7 min at 72ºC in the final cycle. The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5µg/ml) in 1X TBE buffer at 95 volts.

Data Analysis:

PCR products of RAPD markers were separated on 1.5% ethidium bromide-stained agarose gels using 1X TBE buffer running buffer and electrophoresed at 100 volts for 1 h. A 100 bp DNA ladder was used as an indicator for DNA fragment size. Agarose gels were photographed on the gel documentation system. Gels were scored as 0/1 for the absence/presence of DNA fragments, respectively. The total number of bands, the number of polymorphic bands and the percentage of polymorphism were calculated. Data were analyzed using cluster analysis. Similarity matrices were calculated using Jaccard coefficient (Jaccard, 1908).Dendrograms were constructed based on the UPGMA clustering method using NTSYS pc software version 2.0 (Applied Biostatistics, Setauket, New York, USA) (Rohlf et al., 2009).

RESULTS AND DISCUSSION

Measurement of Anatase Titanium Dioxide Nanoparticle size by X-ray Diffractometer (XRD) Technique:

According to Holder and Schaak (2019), the crystallinity and size of the synthesized crystallites of the anatase TiO_2NPs were frequently characterized by the X-ray diffraction (XRD) pattern technique. The structural information was obtained from the comparison of the diffraction pattern of X-rays with the pattern of standardized plane angles of the substance (Irshad *et al.,* 2021).

The particle size and characterization of the materials were checked by the X-ray diffraction (XRD) technique using an X-ray diffractometer (Phillips PW1710, Holland) using The Scherrer Equation at the Metallurgical Development Research Center, Al Tbin, Cairo.

Accordingly, two profiles of X-ray diffraction (XRD) were obtained for the chemically and biologically anatase TiO_2NPs (Fig. 1).

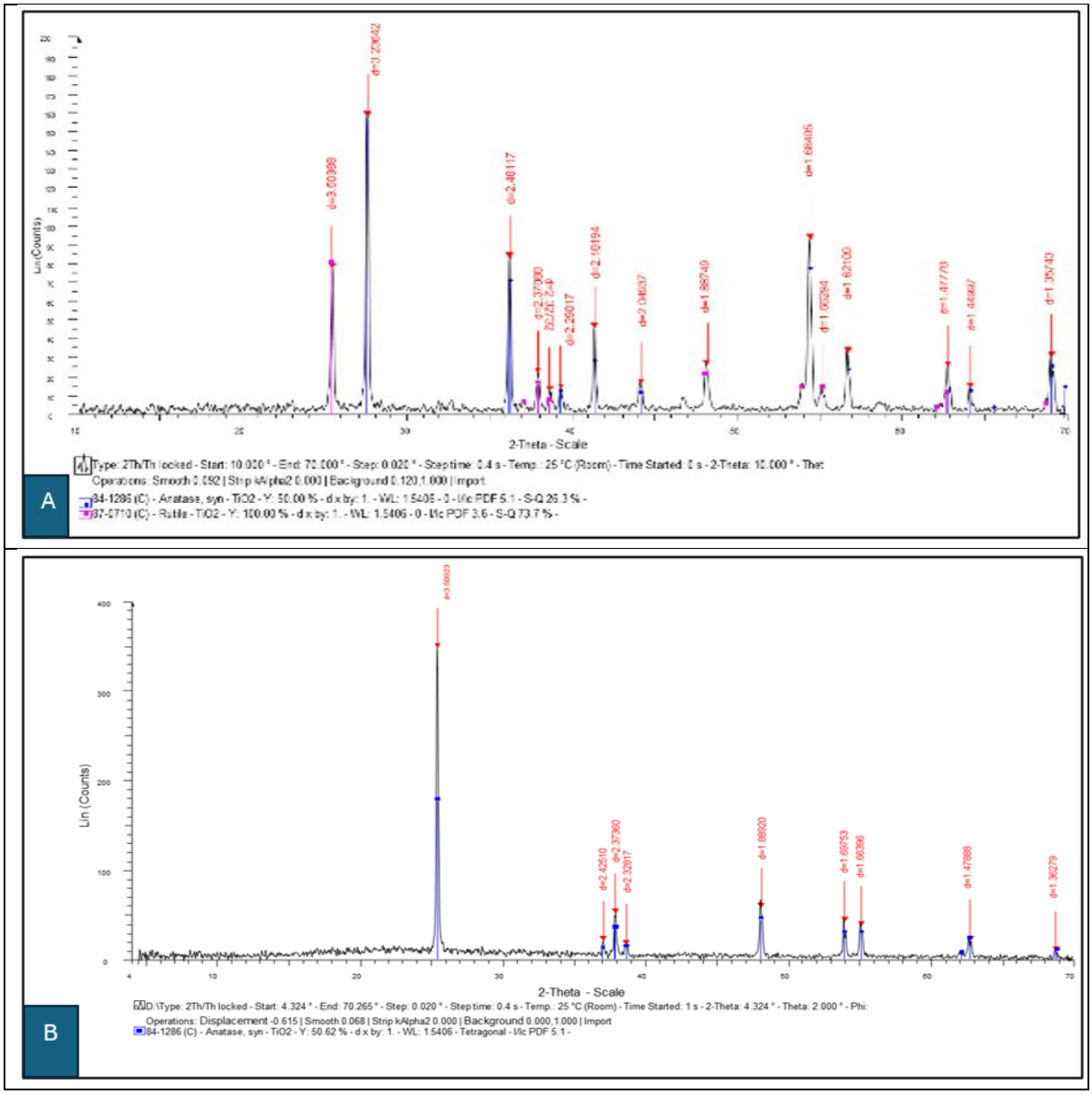


Fig. 1: (A) charted profiles of the X-ray diffraction (XRD) for particle size measurement and characterization of Chemical Anatase Titanium Dioxide Nanoparticles (C-TiO_2NPs). (B): Charted profiles of the X-ray diffraction (XRD) for particle size measurement and characterization of Biological Anatase Titanium Dioxide Nanoparticles (B- TiO_2NPs).

According to the result obtained from the X-ray diffraction (XRD) pattern for particle size measurement and characterization of Chemical Anatase Titanium Dioxide Nanoparticles (C-TiO_2NPs), which were represented in Figure (1 A) and by using Holder and Schaak's equation (2019) for calculation, the C-TiO_2NPs particle size was 15.3 nm. The diminution was the smallest nanoparticle size obtained in the present study, consequently making it the most effective size for entering the mint leaves, and it was expected to have the significantly highest effect on mint photosynthesis, which eventually affects mint physiological metabolism, which was expected to improve mint oil quality and its active ingredient contents. While the obtained particle size of biological Anatase Titanium Dioxide Nanoparticles (B-TiO_2NPs) was 43.7 nm (Fig. 1, B). The diminution was larger than that obtained in the case of C-TiO_2NPs (15.3 nm), but consequently still made it an effective size for entering the mint leaves, and

it was expected to have a significant effect on mint photosynthesis, which eventually affects mint physiological metabolism, which was expected to improve mint oil quality and its active ingredient constituents.

Estimation of Chlorophyll a, Chlorophyll b, Total Chlorophyll, and Total Carotenoids in Spearmint Plants Upon The Application of Biological and Chemical Synthesized TiO_2 NPs:

Data in Table (3) demonstrated the positive impact of TiO_2NPs foliar spray, even from chemical or biological sources on the pigment's contents in spearmint leaves. The application of BNT on spearmint leaves recorded the highest significant increase in chl. a content (22.56 mg/ml) and total chl. content (35.06 mg/ml), it also showed an increase in chl. b content (12.51 mg/ml), while the total carotenoids content (3.24 mg/ml) was significantly decreased compared with the negative control.

In CNT treatments the total carotenoids content was the highest (6.80 mg/ml) significant compared with all treatments, also chl. a, chl. b, and total chl. contents were significantly increased compared with the NC plants with values of (20.13 mg/ml), (10.40 mg/ml), and (30.53 mg/ml) respectively.

Plants that were treated with CNTN and positive control showed a significant increase in all pigment contents (chl. a, chl. b, total chl., and carotenoids) with values of 15.06 mg/ml, 9.53 mg/ml, 24.60 mg/ml, and 6.22 mg/ml respectively for CNTN treated plants and 15.91 mg/ml, 13.44 mg/ml, 29.35 mg/ml, and 5.55 mg/ml respectively for PC plants.

BNTN-treated plants showed a significant increase in chl. a, chl. b, total chl. with values of 18.99 mg/ml, 9.31 mg/ml, and 28.30 mg/ml respectively, while the content of total carotenoids was significantly decreased with a value of 0.34 mg/ml. All these treatments were compared with the negative control plants that have chl. a, chl. b, total chl., and total carotenoids contents with values of 10.82 mg/ml, 4.39 mg/ml, 15.22 mg/ml, and 3.67 mg/ml respectively.

Improvement of spearmint chlorophylls contents upon the application of TiO_2NPs may be due to their photocatalytic effect that promotes the chlorophyll formation by inducing an oxidation-reduction reaction and stimulating the ribulose 1,5-bisphosphate carboxylase activity as supposed by Moaveni *et al.* (2011).

Chaudhary and Singh (2020) also suggested that the Titanium dioxide nanoparticles increase the light absorbance, accelerate the transport and conversion of light energy, protect chloroplasts from aging and prolong the photosynthetic time of the chloroplasts, this may be because of their ability to protect the chloroplasts from excessive light by increasing the activity of antioxidant enzymes, such as catalase, peroxidase, superoxide dismutase. Our results also agreed with Janmohammadi et al., (2016) on barley under supplemental irrigation, Raliya, Nair, et al., (2015) also proved that the foliar application of TiO_2 nanoparticles on tomato (*Solanum lycopersicum* L.) plants can significantly increase the total chlorophyll content and Bilal et al., (2018) as well proved that the foliar application of Titanium dioxide nanoparticles can proficiently improve the chlorophyll and carotenoids contents in peppermint (Mentha piperita) plant.

Table (3): Estimation of Chlorophyll a, Chlorophyll b, Total Chlorophyll and total Carotenoids in spearmint plants treated with chemically, and biologically synthesized TiO_2 nanoparticles, negative and positive controls as well.

Treatments/ Conc.	chl a (mg/ml)	chl b (mg/ml)	Total chl (mg/ml)	Total carotenoids (mg/ml)b
CNT (25 mg/l)	20.13	10.40	30.53	6.80
BNT (50 mg/l)	22.56	12.51	35.06	3.24
NC	10.82	4.39	15.22	3.67
CNTN (25 mg/l)	15.06	9.53	24.60	6.22
BNTN (50 mg/l)	18.99	9.31	28.30	0.34
PC	15.91	13.44	29.35	5.55
LSD 0.05	**3.50**	**1.60**	**4.48**	**1.73**

Where **CNT** is chemically synthesized nano-titanium dioxide, **BNT** is biologically synthesized nano-titanium dioxide, **NC** is Negative control, **CNTN** is chemically synthesized nano-titanium dioxide with nitrogen, **BNTN** is biologically synthesized nano-titanium dioxide with nitrogen, **PC** is positive control, **ƛ** is the wavelength, **chl a:** Chlorophyll a, **chl b:** Chlorophyll b and **Total chl:** Total chlorophyll.

Determination of Micronutrient Contents (µg/g) in Spearmint Leaves as Affected by TiO_2 Nanoparticles Derived From Chemical And Biological Sources as well as Negative and Positive Control:

Unfortunately, many scientists have not analyzed or reported the nutrient concentration of plants after nanoparticle exposure. In fact, the relationship between NPs and plant nutrient uptake can be synergistic, antagonistic, or neutral.

In this study, the effects of TiO_2-NPs from different sources (Biological and Chemical) on micro **(**Fe, Mn, Cu, B, and Zn**)** nutrient elements concentrations of the mint leaves were investigated in Table (4).

The contents of (Fe, Mn, Cu, B, and Zn) micronutrients were the most significantly increased upon the treatment of CNT with values of 11.731 µg/g, 3.023 µg/g, 0.142 µg/g, 0343 µg/g, and 0.279 µg/g respectively. These results were agreed with Lyu et al. (2017), Dağhan, (2018), Gülmezoğlu et al., (2020), and Šebesta et al., (2021), while the treatments of BNT showed that the micronutrient contents of (Fe, Mn, Cu, B, and Zn) were significantly increased with values of 5.042 µg/g, 1.251 µg/g, 0.043 µg/g, 0.143 µg/g, and 0.095 µg/g respectively.

Micronutrients (Fe, Mn, Cu, B, and Zn) in CNTN-treated plants also recorded significantly higher contents than the Negative control with values of 6.164 µg/g, 1.614 µg/g, 0.052 µg/g, 0.168 µg/g, and 0.132 µg/g respectively.

In BNTN-treated plants, the only micronutrients that were significantly increased were Cu and B with values of 0.005 µg/g and 0.023 µg/g, while (Fe, Mn, and Zn) micronutrients were significantly decreased compared with the negative control plants with values of 2.032 µg/g, 0.437 µg/g, and 0.012 µg/g respectively.

For positive control, the micronutrients contents (Fe, Mn, Cu, B, and Zn) were significantly increased with values of 5.292 µg/g, 1.367 µg/g, 0.040 µg/g, 0.136 µg/g, and 0.106 µg/g respectively. All these results were compared with the negative control plants which have values of 2.627 µg/g, 0.580 µg/g, 0.0001 µg/g, 0.016 µg/g, and 0.050 µg/g respectively.

Table 4: Micronutrients content that was affected by the application of TiO2NPs on Mentha spicata L.

Treatment	Fe (µg/g)	Mn (µg/g)	Cu (µg/g)	B (µg/g)	Zn (µg/g)
CNT	11.73	3.023	0.142	0.343	0.279
BNT	5.042	1.251	0.043	0.143	0.095
NC	2.627	0.580	0.0001	0.016	0.050
CNTN	6.164	1.614	0.052	0.168	0.132
BNTN	2.032	0.437	0.005	0.023	0.012
PC	5.292	0.137	0.040	0.136	0.106

Where **CNT** is chemically synthesized nano-titanium dioxide, **BNT** is biologically synthesized nano-titanium dioxide, **NC** is Negative control, **CNTN** is chemically synthesized nano-titanium dioxide with nitrogen, **BNTN** is biologically synthesized nano-titanium dioxide with nitrogen, **and PC** is positive control.

Determination of Mint Oil Constituents by Gas Chromatography-Mass Spectroscopy (GC-MS):

Based on the results of all treatments GC-MS identified 20 important mint oil constituents (Figure 2) with different significant effects. The appearance of some unique compounds in both chemically and biologically synthesized TiO_2NPs and a decrease in the toxic compound isopulegone or its complete disappearance in some treatments compared to the negative control in mint oil indicates a significant improvement in mint oil quality and this was due to their valuable effects (Nair, 2001).

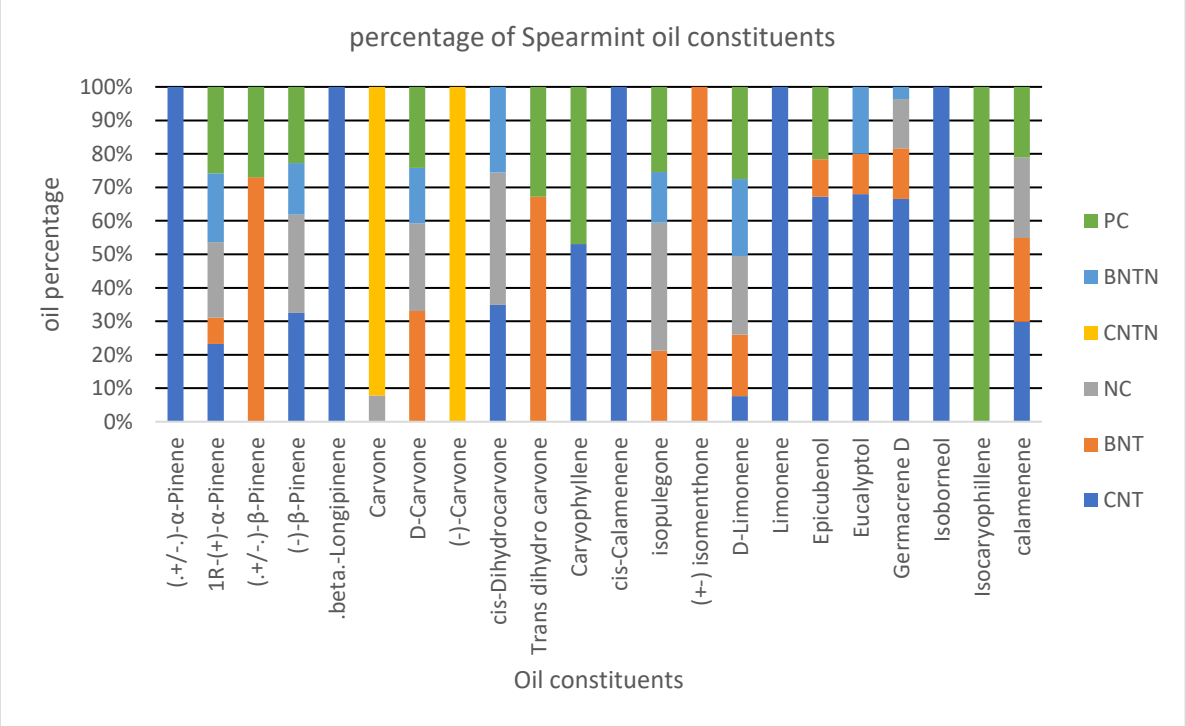


Fig. 2: percentage of Spearmint oil constituents that were identified by GC-MS upon the application of Titanium dioxide nanoparticles on the spearmint plant.

Mint oils have a long history of many uses worldwide, in addition to their therapeutic effects which explains their consideration among the most important essential oils in the world (Fatih et al., 2017). This importance is related to the vital effects

of the oil components, as they are used for many purposes. pinenes, carvones, calamenene, and limonene are within spearmint oil constituents which act as anti-inflammatory (Russo, 2011), antimicrobial (Salehi et al., 2019), anti-viral (Djilani and Dicko, 2012), and anti-bacterial compounds (Limna Mol et al., 2020) (Brahmkshatriya, 2013) (Zhao and Du, 2020). Some detected compounds in mint oil were observed as anti-tumor compounds which could improve the quality of mint oil because they are increased in the treated plants or even appeared in some treatments, while they were absent in the NC. These compounds include β-Pinene, Germacrene D, carvone, limonene, and Epicubenol.

Molecular Analyses:

Polymorphism As Detected by RAPD Analysis:

In this study, a total of 99 bands were amplified among these treatments including 68 monomorphic DNA fragments and 31 were polymorphic. Results of 10 RAPD primers (Table 5 and Figure 3) showed 7 unique markers including 3 positive unique markers (PUMs) and 4 negative unique markers (NUMs). PUMs were characterized by 3 primers from 10 RAPD primers, while negative unique markers (NUMs) were recorded by 4 primers from 10 RAPD primers used in this investigation. These unique bands may be defined as genetic fingerprinting that may be associated with one or more morphological traits. And they may also prove a useful mapping of certain genes that may be associated with an effect of different treatments of Titanium dioxide nanoparticles.

Table 5: Primers used for RAPD analyses and the band characteristics obtained for the six samples of spearmint leaves.

Primers		Total no. of bands	No.of polymorphic bands	No.of monomorphic bands	No. of positive unique markers (PUMs)	No.of negative unique markers (NUMs)	%of polymorphic bands	Total bands amplified	The mean of band frequency
1	OPA-01	8	6	2	--	1	75	35	0.7
2	OPA-03	10	5	5	--	--	50	46	0.8
3	OPA-04	13	1	12	--	--	7.69	74	0.9
4	OPA-05	12	4	8	1	--	33	58	0.8
5	OPA-09	7	4	3	1		57.14	28	0.7
6	OPA-12	11	1	10	--	--	9.09	62	0.9
7	OPB-02	12	3	9	--	1	25	64	0.9
8	OPB-05	8	6	2	1	1	75	30	0.6
9	OPB-08	11	1	10	--	1	9.09	65	1.00
10	OPB-10	7	0	7	--	--	0	42	1.00
Total bands		99	31	68	3	4	341.01	540	
Average		9.9	3.1	6.8	0.3	0.4	31.31	54	

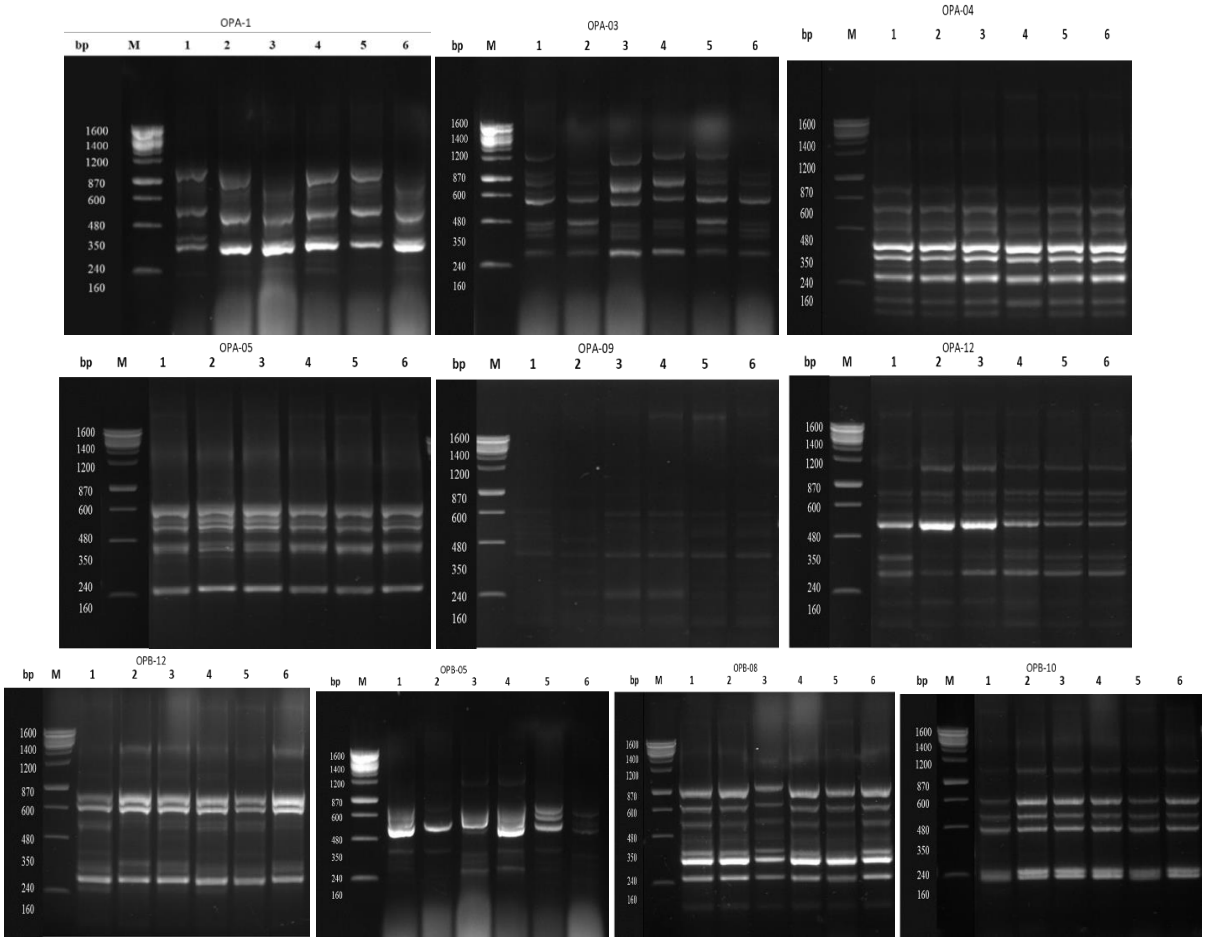


Fig. 3: RAPD pattern generated by primers OPA-01, OPA-03, OPA-04, OPA-05, OPA-09, OPA-12, OPB-02, OPB-05, OPB-08, and OPB-10 Lane M - DNA marker from Chromous Biotcch (100 bp). Where Lane (1): CNT, Lane (2): BNT, Lane (3): NC, Lane (4): CNTN, Lane (5): BNTN, and Lane (6): PC.

Table 6: Nei's genetic identity between the DNA samples of different treatments of Titanium dioxide nanoparticles on Mentha spicata L. from RAPD profile.

	CNT	**BNT**	**NC**	**CNTN**	**BNTN**	**PC**
CNT	**100**					
BNT	89	**100**				
NC	89	92	**100**			
CNTN	91	89	**94**	**100**		
BNTN	92	89	**88**	92	**100**	
PC	90	91	91	92	92	**100**

Where **CNT** is chemically synthesized nano-titanium dioxide, **BNT** is biologically synthesized nano-titanium dioxide, **NC** is Negative control, **CNTN** is chemically synthesized nano-titanium dioxide with nitrogen, **BNTN** is biologically synthesized nano-titanium dioxide with nitrogen and **PC** is positive control.

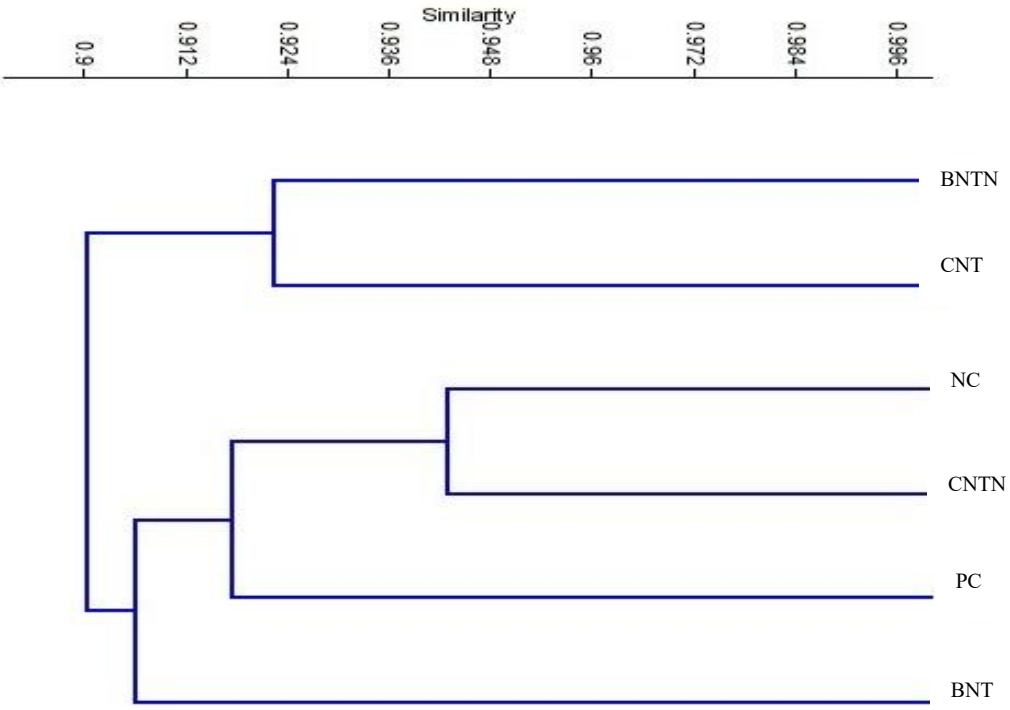


Fig. 4: Dendrogram-based Nei's (1972) genetic distance between the DNA samples of different treatments of Titanium dioxide nanoparticles on *Mentha spicata* L. using UPGMA- modified from NEIGHBOR procedure of PHYLIP Version 3.5. Genomic DNA obtained from CNT (chemically synthesized nano-titanium dioxide), BNT (biologically synthesized nano-titanium dioxide), NC (Negative control), CNTN (chemically synthesized nano-titanium dioxide with nitrogen), BNTN (biologically synthesized nano-Titanium dioxide with nitrogen) and PC (positive control).

RAPD Marker Analysis In Spearmint Samples:

Ten primers were used for the RAPD analysis and these primers showed consistent band patterns. In total, scorable bands were observed with the primers (Table, 5). The total number of bands produced per primer varied from 7 for OPA-09 and OPB-10 to as many as 13 bands for OPA-4, and the average number of bands per primer was 9.9. Out of 99 bands, 31 were polymorphic (31.31%). The average number of polymorphic RAPD bands was 3.1 per primer. The highest similarity (94%) with negative control was recorded in spearmint leaf treated with Titanium dioxide nanoparticles that were chemical source-derived plus nitrogen, while spearmint leaf treated with Titanium dioxide nanoparticles that biological source-derived plus nitrogen was found to show the least similarity (88%) with negative control.

Considering the dendrogram (Fig. 4) constructed from the pooled data, it is evident that the set of primers chosen for the study was able to detect the influence of different treatments on spearmint oil. The estimated similarities relationships ranged from 88 % to 94 %. The highest similarity value (94 %.) was recorded between both NC and CNTN, this indicated that CNTN was closely related to NC. On the other hand, the lowest similarity value (88 %) was recorded between NC and BNTN, indicating that BNTN treatment has a significant effect compared to the NC as shown in Table (6).

The RAPD technique provides an accurate and powerful tool for analyzing genetic variation and relationships. This method detects polymorphism by assaying subsets of the total amount of DNA banding variation in a genome (Kanbar and Kondo, 2011). Wahyudi et al. (2020) proved that

RAPD markers play a successful role in the indication of genetic variation in soybeans.

In the present study, we use RAPD marker to observe the genetic variation of spearmint (*Mentha spicata* L.) upon the application of different treatments of Titanium dioxide nanoparticles, as RAPD is very quick, easy to develop, and more reproducible than other genetic marker such as ISSR and RFLP; and no need to know the background of the genome being analyzed (Kumari and Thakur, 2014). RAPD is a genetic marker that is commonly used for mutation detection (Atienzar and Jha, 2006) and genetic variation studies (Hashem *et al.*, 2018).

The total percent of polymorphism detected by RAPD analysis was 31.31%. This result indicates that there is a genetic difference between the different treatments of n-TIO_2 on Spearmint. Variability in band profiles was observed in this study, where some new bands appeared, and others disappeared. Savva *et al.* (1998) showed that DNA fingerprints will be altered by factors such as exposure of an organism to a genotoxic chemical which results in the formation of a covalently bound adduct between the chemical and the DNA. Faulty repair of these adducts may prevent the primer from binding to those sites command to mutations and, sometimes to cytogenetic changes. Atienzar and Jha, (2006) used RAPD technique and confirmed that when the Taq DNA polymerase encounters a DNA adduct, there are several possible results including blockage, by-pass and the possible dissociation of the enzyme/adduct the complex which will cause changes in RAPD profiles. Breakages that take place in the DNA template between two opposite primers may result in a loss of an amplicon whereas genetic rearrangements and point mutations may be accountable for either a loss or induction of new annealing sites which could result in the disappearance or the appearance of new amplicons, respectively. The appearance of bands could be referring to the presence of new sites that become accessible to primers after structural modulation in DNA sequence that occurred due to mutations (resulting in new annealing events) or large deletions (bringing two pre-existing annealing sites closer) or recombination; while the disappearance of bands may be attributed to the presence of DNA adducts, which can act to block or reduce the polymerization of DNA in the PCR-reaction (Jones and Parry, 1992).

Conclusion:

The present study revealed that Titanium dioxide nanoparticles from biological and chemical sources can significantly improve photosynthetic pigments in *Mentha Spicata* L., additionally, the quality of spearmint oil was improved upon the application of TiO_2 NPs by increasing some therapeutic constituents or appearing new compounds in the oil of CNT, BNT, CNTN treated plants and the positive control. TiO2 NPs also improved the micronutrient contents in spearmint. RAPD analysis was effective in the detection of genetic variation within *Mentha spicata* L. upon the application of different treatments. The similarity matrix also indicated that the most genetic variation was acquired due to the effect of BNTN on spearmint plants as it has the lowest similarity percent (88%) with the negative control followed by the treatment of CNT.

Declarations:

Ethical Approval:Ethical Approval is not applicable.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

Authors Contributions:I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

Funding: No funding was received.

Availability of Data and Materials: All datasets analysed and described during the present study are available from the corresponding author upon reasonable request.

Consent for Publication: Not applicable.
Acknowledgements: Not applicable.

REFERENCES

Atienzar, F. A., and Jha, A. N. (2006). The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. *Mutation Research*, *613*(2–3), 76–102. https://doi.org/10.1016/J.MRREV.2006.06.001

Ay Kee, L., Bakr Shori, A., and Salihin Baba, A. (2017). Bioactivity and health effects of Mentha spicata. *Integrative Food, Nutrition and Metabolism*, *5*(1). https://doi.org/ 10.15761/IFNM.1000203

Bilal, A., Shabbir, A., Jaleel, H., Khan, M. M. A., and Sadiq, Y. (2018). Efficacy of Titanium dioxide nanoparticles in modulating photosynthesis, peltate glandular trichomes and essential oil production and quality in Mentha piperita L. *Current Plant Biology*, *13*(4), 6–15. https://doi.org/10.1016/j.cpb.2018.04.002

Brahmkshatriya, P.P., Brahmkshatriya, P.S. (2013). Terpenes: Chemistry, Biological Role, and Therapeutic Applications. In: Ramawat, K., Mérillon, JM. (eds) Natural Products. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-22144-6_120

Bussler, W. (1972). Epstein, E.: Mineral Nutrition of Plants: Principles and Perspectives. John Wiley and Sons, Inc., New York, London, Sydney, Toronto. 1972. *Zeitschrift Für Pflanzenernährung Und Bodenkunde*,132 (2),158–159.https://doi.org/10.1002/JPLN.19721320211

Chaudhary, I., and Singh, V. (2020). Titanium Dioxide Nanoparticles and its Impact on Growth, Biomass and Yield of Agricultural Crops under Environmental Stress: A Review. *Research Journal of Nanoscience and Nanotechnology*, *10*(1), 1–8. https://doi.org/10.3923/rjnn.2020.1.8

Coleman, W. B., and Tsongalis, G. J. (2005). The polymerase chain reaction. *Molecular Diagnostics: For the Clinical Laboratorian*, 47–55. https://doi.org/10.1385/1-59259-928 -1:047/COVER

Dağhan, H. (2018). Effects of tio2 nanoparticles on maize (Zea mays L.) growth, chlorophyll content and nutrient uptake. *Applied Ecology and Environmental Research*, *16*(5), 6873–6883. https://doi.org/10.15666/aeer/1605_68736883

Djilani, A., and Dicko, A. (2012). The Therapeutic Benefits of Essential Oils. In Nutrition, Well-Being and Health. InTech. 7: pp. 155–179. https://doi.org/10.5772/25344

Duncan, D.B. (1955) Multiple Range and Multiple F-Test. *Biometrics,* 11, 1-5

Emadpour, M., Hosseini, R., and Beiramizadeh, E. (2009). A high genetic diversity revealed between commercial rose cultivars by RAPD-PCR technique. In *International Journal of Plant Production* (Vol. 3, Issue 4). Online.

Fakruddin, M., Hossain, Z., and Afroz, H. (2012). Prospects and applications of nanobiotechnology: A medical perspective. *Journal of Nanobiotechnology*, 10 (1), 1–8. https://doi.org/10.1186/1477- 3155-10 -31/METRICS

Fatih, B., Madani, K., Chibane, M., and Duez, P. (2017). Chemical Composition and Biological Activities of Mentha Species. *Aromatic and Medicinal Plants - Back to Nature*. https://doi.org/10.5772/67291

Gülmezoğlu, N., Köleli, N., Karakaya, B., and Dağhan, H. (2020). Impact of Titanium dioxide nanoparticles (TiO2-NPs) on growth and mineral nutrient uptake of wheat (*Triticum vulgare* L.). *Tarla Bitkileri Merkez Arastirma Enstitusu*, *29*(2), 69–76. https://doi.org/10.38042/BIOST.202

0.29.02.03

Hashem, M., Rizk, I., and Abd El-Azeem, R. (2018). Genetic variations among two Egyptian mint species (Mentha spicata and Mentha piperita) using random amplified Polymorphic DNA markers. *Research Journal of Applied Biotechnology*, *4*(2), 1–8. https://doi.org/10.21608/rjab.2018.106784

Holder, C. F., and Schaak, R. E. (2019). Tutorial on Powder X-ray Diffraction for Characterizing Nanoscale Materials. ACS Nano 2019 13 (7), 7359-7365 DOI: 10. 1021/acsnano.9b05157

Ibrahim, H. M. M. (2017). Assessment of Genetic Diversity and Relationships of Five *Mentha* Species Using RAPD Marker. *Current Science International*, 6 (2), 271–277.

Irshad, M. A., Nawaz, R., Rehman, M. Z. ur, Adrees, M., Rizwan, M., Ali, S., Ahmad, S., and Tasleem, S. (2021). Synthesis, characterization and advanced sustainable applications of Titanium dioxide nanoparticles: A review. *Ecotoxicology and Environmental Safety*, 212: 111978. https://doi.org/10.1016/J.ECOENV.2021.111978

Jaccard, P. (1908) Nouvelles researches sur la distribution florale. *Bulletin de la Société vaudoise des sciences naturelles, 44, 223-270. - References - Scientific Research Publishing*, Retrieved May 27, 2023, from https://www.scirp.org/ (S(351jmbntvnsjt1aadkposzje))/reference/referencespapers.aspx?referenceid=66293

Janmohammadi, M., Amanzadeh, T., Sabaghnia, N., and Dashti, S. (2016). Impact of foliar application of nano micronutrient fertilizers and Titanium dioxide nanoparticles on the growth and yield components of barley under supplemental irrigation. *Acta Agriculturae Slovenica*, *107*(2), 265–276. https://doi.org/10.14720/aas.2016.107.2.01

Jha, A. K., Prasad, K., and Kulkarni, A. R. (2009). Synthesis of TiO2 nanoparticles using microorganisms. *Colloids and Surfaces B: Biointerfaces*, *71*(2), 226–229. https://doi.org/10.1016/j.colsurfb.2009.02.007

Jones, N. J., and Parry, J. M. (1992). The detection of DNA adducts, DNA base changes and chromosome damage for the assessment of exposure to genotoxic pollutants. *Aquatic Toxicology*, 22(4), 323–343. https://doi.org/10.1016/0166-445X(92)90048-R

Kabir, S., Tahira, R., Khan, M. F., Naeemullah, M., and Jamal, A. (2014). Molecular Characterization of Genetic Diversity of Mint Gene Pool through RAPD Markers. *International Journal of Agriculture Innovations and Research*, *2*(4), 2319–1473.

Kanbar, A., and Kondo, K. (2011). Efficiency of ISSR and RAPD dominant markers in assessing genetic diversity among Japanese and Syrian cultivars of barley (H. vulgare). *Research Journal of Agriculture and Biological Sciences*, 7(1):4-10. https://www. researchgate .net/publication/259527711

Kleiber, T., & Markiewicz, B. (2013). Application of "Tytanit" in greenhouse tomato growing. Acta Scientiarum Polonorum-hortorum Cultus, 12, 117-126.

Kumari, N., and Thakur, S. K. (2014). Randomly Amplified Polymorphic Dna-A Brief Review. *American Journal of Animal and Veterinary Sciences*, 9(1), 6–13. https://doi.org/10.3844/ajavssp.2014.6.13

Limna Mol, V. P., Abdulaziz, A., Sneha, K. G., Praveen, P. J., Raveendran, T. V., and Parameswaran, P. S. (2020). Inhibition of pathogenic *Vibrio harveyi* using calamenene, derived from the Indian *gorgonian*

Subergorgia reticulata, and its synthetic analog. 3 Biotech (2020) 10:248. https://doi.org/10.1007/s13205-020-02241-1

Lyu, S., Wei, X., Chen, J., Wang, C., Wang, X., and Pan, D. (2017). Titanium as a beneficial element for crop production. *Frontiers in Plant Science*, *8*(4), 1–19. https://doi.org/10.3389/fpls.2017.00597

Moaveni, P., Talebi, A., Farahani, H. A., and Maroufi, K. (2011). Study of Nano Particles TiO2 Spraying on Some Yield Components in Barley (*Hordem Vulgare* L .) 15: 115-119.

Nair B. Final report on the safety assessment of Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Leaf Extract, Mentha Piperita (Peppermint) Leaf, and Mentha Piperita (Peppermint) Leaf Water. Int J Toxicol. 2001;20 Suppl 3:61-73. PMID: 11766133.

Nei, M. (1972). Genetic distance between populations. *American Naturalist*, *106*(949), 283–292. https://doi.org/10.2307/2459777

Porebski, S., Bailey, L. G., and Baum, B. R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter*, *15*(1), 8–15. https://doi.org/10.1007/ BF 02772108

Raliya, R., Nair, R., Chavalmane, S., Wang, W.-N., and Biswas, P. (2015). Mechanistic evaluation of translocation and physiological impact of Titanium dioxide and zinc oxide nanoparticles on the tomato (Solanum lycopersicum L.) plant †. *Metallomics*, *7*: 1584. https://doi.org/10.1039/c5mt00168d

Rohlf, F. J., Applied Biostatistics, Inc., and Exeter Software (Firm). (2009). *NTSYS-pc :* numerical taxonomy and multivariate analysis system. https://www.researchgate.net/publication/246982444_NTSYS-pc_Numerical Taxonomy and Multivariate Analysis System.

Russo, E. B. (2011). Taming THC: Potential cannabis synergy and phytocannabinoid- terpenoid entourage effects. *British Journal of Pharmacology*, *163*(7), 1344–1364. https://doi.org/10.1111/j.1476-5381.2011.01238.x

Sabri, H. Y., and Saeed, A. H. (2023). Estimation of Genetic Distance Using RAPD Markers for Five Varieties of *Citrullus lanatus* L. *IOP Conference Series: Earth and Environmental Science*, 1214(1).

Sadak, M.S. (2019). Impact of silver nanoparticles on plant growth, some biochemical aspects, and yield of fenugreek plant (*Trigonella foenum-graecum*). Bulletin of the National Research Centre 43, 38. https://doi.org/10.1186/s42269-019-0077-y

Salehi, B., Upadhyay, S., Orhan, I. E., Jugran, A. K., Jayaweera, S. L. D., Dias, D. A., Sharopov, F., Taheri, Y., Martins, N., Baghalpour, N., Cho, W. C., and Sharifi-Rad, J. (2019). Therapeutic Potential of α- and β-Pinene: *A Miracle Gift of Nature. Biomolecules,* 9 (11), 738. https://doi.org/10.3390/BIOM9110738

Savva, D. (1998). Use of DNA fingerprinting to detect genotoxic effects. Ecotoxicology and Environmental Safety, 41(1), 103–106. https://doi.org/10.1006/EESA.1998.1674

Šebesta, M., Ramakanth, I., Zvěřina, O., Šeda, M., Diviš, P., and Kolenčík, M. (2021). Effects of Titanium Dioxide Nanomaterials on Plants Growth. *Nanotechnology in Plant Growth Promotion and Protection*, 17–44. https://doi.org/10.1002/9781119745884.CH2

Shabbir, A., Khan, M. M. A., Ahmad, B., Sadiq, Y., Jaleel, H., and Uddin, M. (2019). Efficacy of TiO2 nanoparticles in enhancing the photosynthesis, essential oil and khusimol biosynthesis in *vetiveria*

zizanioides L. Nash. *Photosynthetica*, 57(2), 599–606. https://doi.org/10.32615/ps.2019.071

Shafi, A., Qadir, J., Sabir, S., Zain Khan, M., and Rahman, M. M. (2020). Nanoagriculture: A Holistic Approach for Sustainable Development of Agriculture. In Handbook of Nanomaterials and Nanocomposites for Energy and Environmental Applications (pp. 1–16). Springer International Publishing. https://doi.org/10.1007/978-3-030-11155-7_48-1

Siddiqui, M. H., Al-Whaibi, M. H., Firoz, M., and Al-Khaishany, M. Y. (2015). Role of nanoparticles in plants. *Nanotechnology and Plant Sciences: Nanoparticles and Their Impact on Plants*, 19–35. https://doi.org/10.1007/ 978-3-319-14502-0_2/ FIGURES /1

Soliman, M. S., Elhalafawi, K., Ibrahim, I. A., Mahrous, E., and Bekhit, M. (2018). Utilizing RAPD Analysis as Molecular Markers for comparison between Various Callus and Mother Plant of *Jatropha curcas* L. *In Research Journal of Applied Biotechnology (RJAB) Research Journal of Applied Biotechnology*, 4 (2),75-85.

Straumite, E., Kruma, Z., and Galoburda, R. (2015). Pigments in mint leaves and stems. In *Agronomy Research* (Vol. 13, Issue 4).

Sumanta, N., Imranul Haque, C., Nishika, J., and Suprakash, R. (2014). Spectrophotometric Analysis of Chlorophylls and Carotenoids from Commonly Grown Fern Species by Using Various Extracting Solvents. *Research Journal of Chemical Sciences*, *4*(9), 63–69. www.isca.me

Tripathi, D. K., Shweta, Singh, S., Singh, S., Pandey, R., Singh, V. P., Sharma, N. C., Prasad, S. M., Dubey, N. K., and Chauhan, D. K. (2017). An overview on manufactured nanoparticles in plants: Uptake, translocation, accumulation and phytotoxicity. *Plant Physiology and Biochemistry*, 110 :2–12. https://doi.org/10.1016/J.PLAPHY.2016.07.030

Tucker A.O. Mentha: Economic uses. In: Lawrence B.M., editor. Mint: The Genus Mentha. CRC Press, Taylor & Francis Group; Boca Raton, FL, USA: 2007. pp. 519–522.

Wahyudi, D., Hapsari, L., and Sundari. (2020). RAPD Analysis for Genetic Variability Detection of Mutant Soybean (*Glycine max* (L.) Merr). *Journal of Tropical Biodiversity and Biotechnology*, *5*(1), 68–77. https://doi.org/10.22146/JTBB.53653

Zhao, M., and Du, J. (2020). Anti-inflammatory and protective effects of D-carvone on lipopolysaccharide (LPS)-induced acute lung injury in mice. *Journal of King Saud University - Science*, *32*(2), 1592–1596. https://doi.org/10.1016/j.jksus.2019.12.016