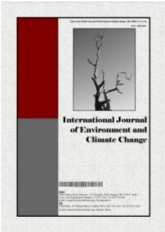

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Biochemical Characteristics of Graphene and Titanium Dioxide Nanoparticles in Okra (*Abelmoschus esculentus* L.)

Pinninty Sandhya Reddy [aφ], Sadras Bhavana [aφ] and Pradeep K. Shukla [b*φ]

[a] *Department of Crop Physiology, School of Agricultural Sciences, Mallareddy University, Hyderabad, India.*
[b] *Department of Biological Sciences, Faculty of Science, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj-211007, U.P., India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A study on biochemical characteristics of graphene and titanium dioxide in two varieties of okra (NAYAN-11 and STAR-77) was conducted during the zaid season of 2018. A pot experiment was carried out on the research field of the department of Biological Sciences with five treatments using varying quantities of graphene nanoparticles (500 ppm and 1000 ppm) and TiO_2 nanoparticles (25 ppm, 50 ppm and 100 ppm) along with a control. The experiment was planned using four replications. The layout used was complete randomized block design. Purpose of the study was to analyse biochemical characteristics of okra under the varied nanoparticle treatments. The experiment stated that the graphene and TiO_2 nanoparticles were advantageous to okra crop in terms of yield. Among the treatments, 100 ppm of TiO_2 nanoparticles were found to be favouring the biochemical and yield parameters in both the varieties of okra. As compare to the STAR-77 variety NAYAN-11 showed better performance in respect to biochemical characteristics and yield.

Keywords: Graphene; TiO_2; biochemical parameters; okra.

[φ] *Assistant Professor;*
**Corresponding author: E-mail: pradeep.shukla@shiats.edu.in;*

1. INTRODUCTION

Nanotechnology is an expeditiously developing and exhilarating subject of science that sanctions for advanced research in many fields and brings up incipient opportunities for biotechnology and agricultural applications. Crop development and yield may be significantly ameliorated by the integration of nanoparticles to plants [1-3]. Although fertilizers are essential for the functional biology and development of plants, the majority of applied fertilizers remain inaccessible to plants due to a variety of processes, including leaching, photolysis, hydrolysis and decomposition. Consequently, it is essential to minimize fertilization losses and boost crop engenderment by utilizing novel application strategies with the avail of nanotechnology and nanomaterials. Nanotechnology is an expeditiously developing and exhilarating subject of science that sanctions for advanced research in many fields and brings up incipient opportunities for biotechnology and agricultural applications. Crop development and yield may be significantly amended by the integration of nanoparticles to plants. Nanoparticles (withal recognized as nanoscale particles, or NSPs) are atomic or molecular aggregates having at least one dimension between 1-100 nm [4], which can significantly alter their physico-chemical characteristics in comparison to the bulk material [5].

"The desideratum of research in nanotechnology commenced growing for industrial applications proximately half a century ago, the drive for utilization of nanotechnology in agriculture came only with recently published reports. It has been envisioned that the novel properties of nanoscale biomaterials cumulated with ingenious engineering would have innovative applications for agriculture and food industries" [6-13]. The creation of tools for diagnostics and regulated drug delivery has opened up several avenues for interaction between the domains of biology and nanotechnology in recent years. On the other hand, there has been less contact between the two fields in the area of plant biology. The majority of the published research in this area focuses on the environmental effects of nanoparticles on crop growth and magnification, as well as on the bio-engineering of nanoparticles using plant extracts [14].

Due to their abilities to influence seed germination, seedling multiplication, and plant growth in a variety of plant species, carbon nanotubes, or graphene, which are known for their light weight, prodigious vigour, and extraordinary conductivity, have a special position in agricultural applications [15,16].

Due to incremented metabolic activity and better nutrient utilization of native nutrients by stimulating microbial activities, titanium dioxide NPs show promise as an efficacious nutrient source for plants to boost biomass output [17]. TiO_2 enhances metabolic processes and the synthesis of proteins and carbohydrates, which amends plant output [18]. In view of the aforementioned facts, a trial was conducted to understand roles of graphene and titanium dioxide nanoparticles on the biochemical traits and yield of two okra cultivars.

2. MATERIALS AND METHODS

The experiment was conducted in the *zaid* season in the Department of Biological Sciences, SHUATS, Allahabad, Uttar Pradesh, which is situated at 25° 24' 42" N, 81° 50' 56" E, and 98 m above mean sea level. The subtropical climate of Allahabad is prevalent. The winter season is extremely cold, with lows of 2.5°C, while the summer season is extremely hot, with highs of 48°C. Five treatments including four replications were used for CRD design. For the experiment, seeds from the okra cultivars NAYAN-11 and STAR-77 were treated with five concentrations of nanoparticles, including T_0 (control), T_1 (500 ppm graphene), T_2 (1000 ppm graphene), T_3 (25 ppm TiO_2), T_4 (50 ppm TiO_2), and T_5 (100 ppm TiO_2).

The various biochemical characteristics like chlorophyll 'a', chlorophyll 'b' contents, carotenoids content, protein and carbohydrates content were estimated using standard methods as given below.

2.1 Chlorophyll Content (mg/g FW)

The chlorophyll content was calculated in accordance with Lichtenthaler and Wellburn [19]. 1 g of leaves were weighed, crushed in 80% acetone to make 10 ml, then centrifuged at 800 ppm for five minutes. A reading of the supernatant was taken at 663, 645 nm. The measurements were entered into the formula below, and the results were calculated using a spectrophotometer. Chlorophyll contents was calculated by using the following formula and expressed in mg/g fresh weight^{-1}:

$$\text{Chlorophyll 'a' (mg/g)} = 12.7 \times (A663) - 2.69 \times (A645) \times \frac{V}{1000 \times w \times a}$$

$$\text{Chlorophyll 'b' (mg/g)} = 22.9 \times (A645) - 4.68 \times (A663) \times \frac{V}{1000 \times w \times a}$$

$$\text{Total chlorophyll (mg/g)} = 20.2 \times (A645) + 8.02 \times (A663) \times \frac{V}{1000 \times w \times a}$$

Where,

A645 = Absorbance of the extract at 645 nm
A663 = Absorbance of the extract at 663 nm
a = Path length of cuvette (1 cm)
V = Final volume of the chlorophyll extract (10 ml)
W = Fresh weight of the sample (0.10 g)

2.2 Carotenoid Content (mg/g FW)

Carotenoid was determined according to Lin and Wellborn (1983). 0.5 gm and homogenized in 10 ml of acetone (80% acetone). Next to the centrifuged at 3000 rpm at 10 min. The absorbance was recorded at 470 nm.

It was calculated by the formula –

Total carotenoids = [1000A470- (3.27 Chl-a+104 Chlb)]/22

2.3 Protein (%)

2.3.1 Protein was estimated by Lowry's method

Protein concentration was determined by pipetting 50 µl of supernatant, comprising proteins. The sampling was done into test tubes in duplicates of three, with a total volume of 1 ml. As a blank, a tube containing 1 ml of distilled water was used. Each tube, including the blank, received 3 ml of reagent C. After thoroughly mixing the solution, the solution was allowed to stand for 30 min. Next, 0.5 ml of reagent D was added. The tubes were then kept at room temperature in the dark for 60 min. The solution took on the blue colour. In a UV-visible spectrophotometer, the absorbance was measured at 660 nm. Using a standard graph, the sample's protein content was determined and represented as mg/g.

2.4 Carbohydrate (g)

The total carbohydrate content was estimated by using the method given by Hedge and Hofreiter [20].

100 mg of the leaf samples were added to 5.0 ml of 2.5 N HCl, then kept in a boiling water bath for three hours and then cooled to room temperature. When the effervescence stopped, neutralised with solid $NaCO_3$ until the volume reached 100 ml, centrifuged, collected the supernatant, and took 0.2 to 1.0 ml for analysis. 0.2 to 1 ml of the working standards were used to prepare the standards. As a blank, 1.0 ml of distilled water was used to fill all the tubes to a volume of 1.0 ml. 4.0 ml of anthrone reagent was then added, heated for eight minutes in a boiling water bath, quickly cooled, and the green to dark green colour was read at 630 nm.

2.5 Calculation

A standard graph was drawn by taking the concentration of glucose on X axis and spectrophotometer reading on Y axis. From the graph the concentration of glucose in the sample was calculated.

2.6 Yield per Plant

The total number of fruits from plants was counted manually for each treatment. Average was worked out and recorded as number of fruits per plant.

3. RESULTS AND DISCUSSION

Various biochemical parameters *viz*., chlorophyll content, carotenoids, protein and carbohydrates were measured in leaf samples of two varieties of okra. The observations made were analysed using CRD statistical design and the results showed that the two varieties of okra performed variedly at different concentrations of grapheme and titanium dioxide.

3.1 Chlorophyll Content

The two varieties *viz*., Nayan-11 and Star-77 have showed more amounts of chlorophyll content (chl-a, chl-b and total chlorophylls) at 100ppm of TiO_2 treatment (3.07, 3.2 and 2.2 mg/g FW) and were on par with the remaining treatments. The minimum concentration of chlorophyll-a were observed for the treatment 50ppm of TiO_2 (2.86 mg/g) in Nayan-11 and 1000ppm of graphene (2.55 mg/g) in Star-77. Similarly, the minimum concentrations of chlorophyll-b were recorded at T_0 (control) and 500ppm of graphene in two varieties (Nayan-11 and Star-77) respectively. However the minimum total chlorophyll content was recorded at 25 ppm TiO_2 and T_0 (control) for the varieties Nayan-11 and Star-77 respectively.

Table 1. Effect of graphene and titanium dioxide nanoparticles on biochemical characteristics in leaf tissue of okra varieties (NAYAN-11 and STAR77)

Treatments	Chlorophyll-a (mg/g fresh weight)		Chlorophyll-b (mg/g fresh weight)		Total Chlorophyll (mg/g fresh weight)		Carotenoids (mg/g fresh weight)		Protein (%)		Carbohydrate (%)	
	NAYAN-11	STAR-77	NAYAN-11	STAR-77	NAYAN-11	STAR-77	NAYAN-11	STAR-77	NAYAN-11	STAR-77	NAYAN-11	STAR-77
T0 (control)	2.93	3.02	2.11	1.91	2.10	1.89	10.80	10.46	0.31	0.22	1.73	1.37
T1 (500 ppm graphene)	3.06	2.93	2.68	1.81	2.11	2.11	11.09	10.89	0.17	0.28	1.43	1.79
T2 (1000 ppm graphene)	2.97	2.55	2.34	2.53	2.21	2.18	11.15	10.51	0.25	0.33	1.49	1.96
T3 (25 ppm TiO_2)	2.93	2.74	2.29	1.95	2.05	2.00	10.50	10.08	0.31	0.25	1.76	2.14
T4 (50 ppm TiO_2)	2.86	2.99	2.63	2.21	2.21	2.12	10.53	10.23	0.33	0.33	1.90	2.33
T5 (100 ppm TiO_2)	3.07	3.06	3.21	2.73	2.22	2.20	11.37	11.20	0.54	0.40	2.35	2.34
Mean	2.97	2.88	2.54	2.19	2.15	2.08	10.91	10.56	0.32	0.30	1.77	1.99
C.V.	3.94	5.51	24.27	21.50	3.73	5.41	14.32	13.50	32.80	44.45	29.66	33.34
S.E	0.06	0.08	0.31	0.24	0.04	0.06	0.78	0.71	0.05	0.07	0.26	0.33
C.D.5%	0.17	0.24	0.92	0.70	0.12	0.17	2.32	2.12	0.16	0.20	0.78	0.99

Table 2. Effect of graphene and titanium dioxide dioxide nanoparticles on yield (fruits/plant) in okra varieties. (NAYAN-11 and STAR-77)

	Yield(fruits/plant)	
Treatments	**NAYAN-11**	**STAR-77**
T0	6.75	3.50
T1	7.00	3.50
T2	8.50	5.00
T3	6.25	3.75
T4	7.00	5.00
T5	10.00	6.50
Mean	7.58	4.54
C.V.	22.95	27.34
S.E	0.87	0.62
C.D.5%	2.59	1.85

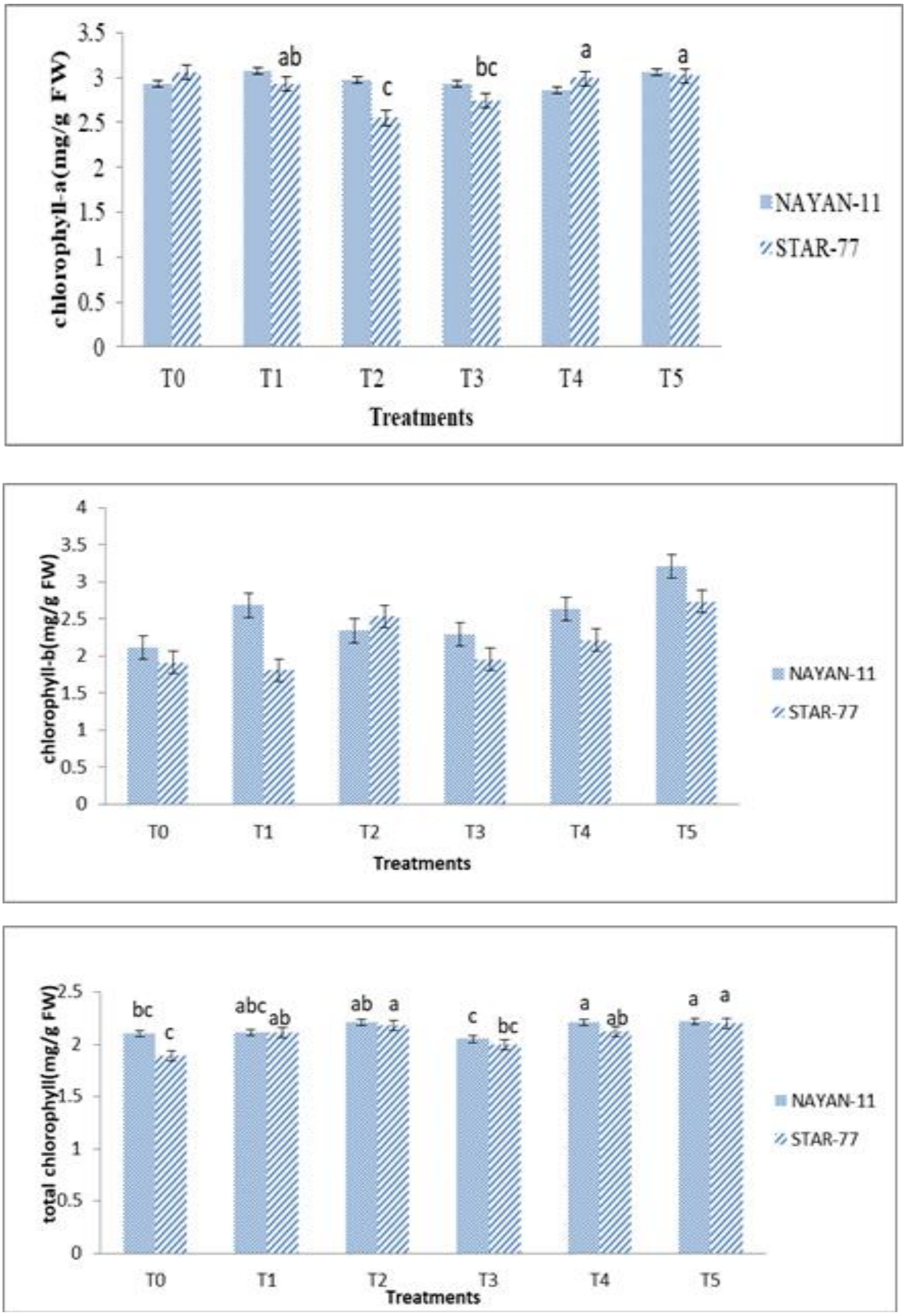

Fig. 1. Effect of different concentration of graphene and titanium dioxide nanoparticles on chlorophyll-a, chlorophyll-b and total chlorophylls (mg/g) in leaf tissue of okra varieties (NAYAN-11 and STAR-77)

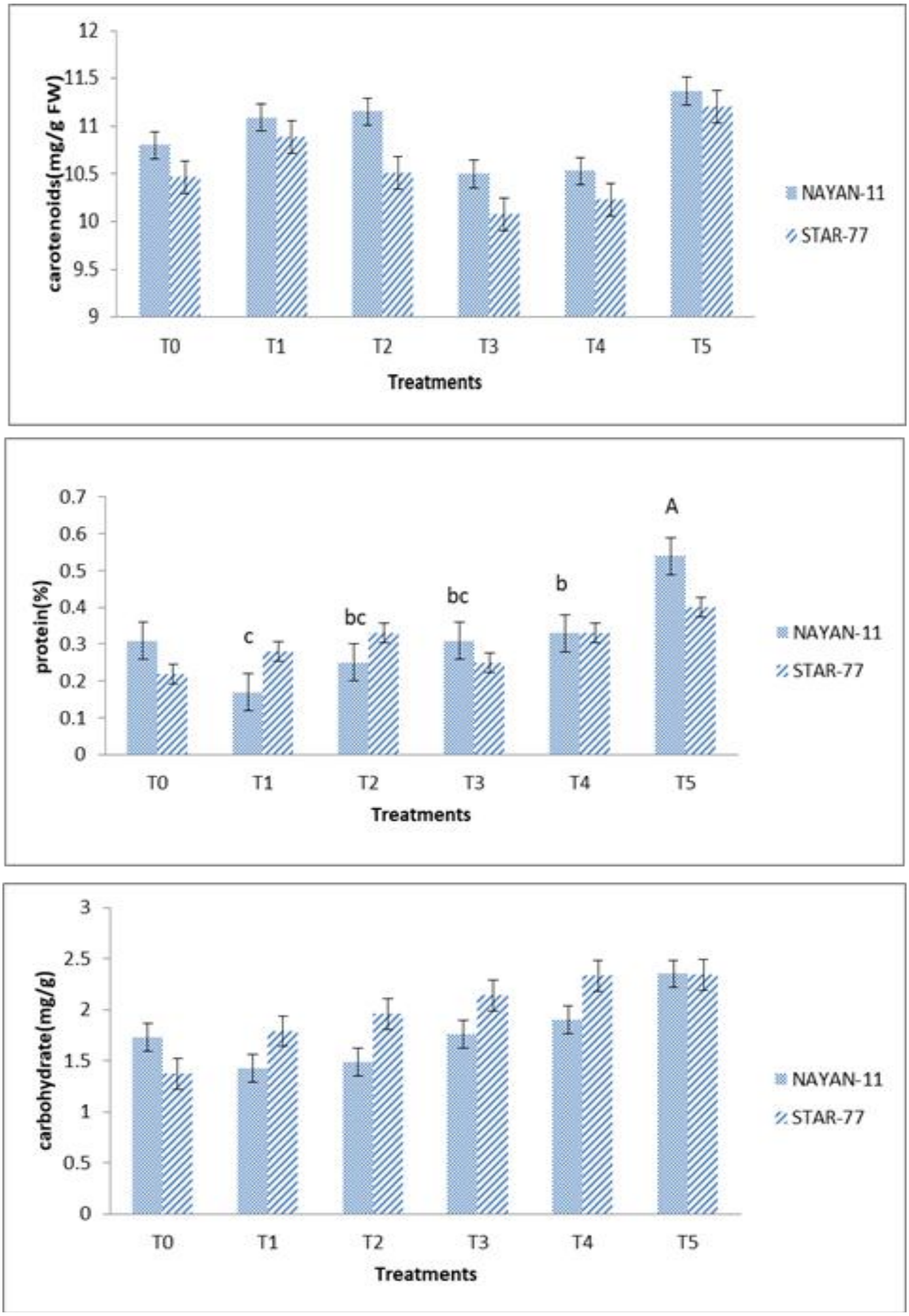

Fig. 2. Effect of different concentration of graphene and titanium dioxide nanoparticles on carotenoids (mg/g), protein (%) and carbohydrates (g) in leaf tissue of okra varieties (NAYAN-11 and STAR-77)

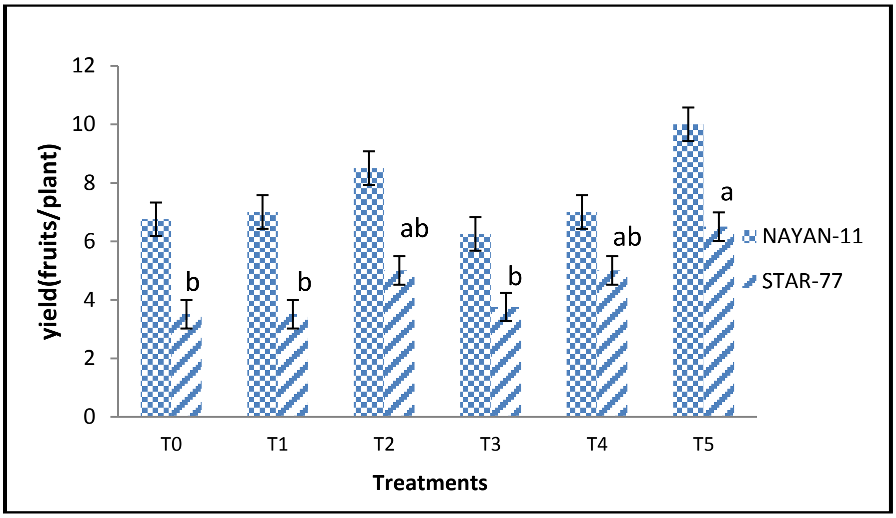

Fig 3. Effect of different concentration of graphene and titanium dioxide nanoparticles on yield (fruits/plant) in okra varieties. (NAYAN-11 and STAR-77)

The Ribulose-1,5-bisPhosphate Carboxylase (Rubisco) activity was stimulated and photosynthesis was increased [21] by the 100 ppm TiO_2 NPs, leading to an increase in plant growth and development. TiO_2 NPs shield the chloroplast against ageing for a prolonged period [22]. Similar findings were reported by Zheng et al. [23], who found that nano-TiO_2 boosted photosynthetic carbon absorption by activating (Rubisco), which might accelerate rubisco carboxylation and hence increase plant development.

3.2 Carotenoids

The concentrations of carotenoids have shown significant variation among the different treatments of graphene and TiO_2. The maximum levels of carotenoids (11.3 mg/g FW) were observed at 100 ppm of TiO_2 while, the minimum levels of carotenoids were recorded at 25 ppm of TiO_2 (10.5 &10.08 mg/g FW) in both the varieties of okra (Nayan-11 and Star-77).

3.3 Protein Content

The protein percentage recorded in both the varieties of okra showed a significant difference. The maximum protein % was observed at 100 ppm of TiO_2 (0.54% & 0.40%) and minimum protein % was observed at 500 ppm graphene and T_0 (control) (0.17 & 0.22) in two varieties of okra respectively (Nayan-11 & Star-77). "TiO_2 NPs controls the activity of enzymes involved in the metabolism of nitrogen, including nitrate reductase, glutamate dehydrogenase, glutamine synthase, and glutamic-pyruvic transaminase, which aids in nitrate absorption by plants and favours the conversion of inorganic nitrogen to organic nitrogen in the form of protein and chlorophyll, which may increase plant fresh weight and dry weight" [24].

3.4 Carbohydrates

Significant difference was recorded for the carbohydrate content analysed in two varieties of okra. The maximum carbohydrates were shown by 100 ppm of TiO_2 treatment (2.35 mg/g) while, the minimum was recorded by T_0 (control) (1.43 & 1.37 mg/g) in both the varieties of okra. The increase in chlorophyll content gradually enhanced the photosynthetic activity in okra which resulted in effective increase of carbohydrates at 100 ppm of TiO_2 treatment. According to Chao et al. [18] application of 100 ppm TiO_2 NPs to food crops has been shown to boost plant development, increase photosynthetic rate, lessen the severity of disease, and increase output by 30%.

3.5 Yield Parameters

The yield component was recorded for all the treatments and control where the maximum fruits/plant was shown by 100ppm of TiO_2 treatment for both the varieties of okra. Different concentrations of graphene also showed (1000 ppm graphene NPs) better result for the biochemical characters like chlorophyll content, carotenoids, protein, and carbohydrates. But the response showed by graphene was minimal than the response showed by TiO_2 nanoparticles.

4. CONCLUSION

It can be concluded that the higher concentration of graphene and TiO_2 nanoparticles were advantageous to the yield of okra crops. Among the treatments 100 ppm TiO_2 was found to be most favouring to biochemical and yield parameters in both the varieties of okra. Among the varieties NAYAN-11 showed better performance for biochemical and yield parameters than STAR-77.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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