EFFECT OF GRAPHENE AND TITANIUM DIOXIDE NANOPARTICLES ON THE BIOCHEMICAL CHARACTERISTICS IN OKRA (*Abelmoschus esculentus* L.)

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 summer season of 2018, at department of Biological Sciences, Sam Higginbottom University of Agriculture, Technology & Sciences, Uttar Pradesh. The pot experiment was carried out with six treatments i.e, different concentrations of graphene (500 ppm and1000 ppm) and TiO_2 nanoparticles (25 ppm, 50 ppm and 100 ppm) along with control in 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.

Key words: Graphene, TiO_2, biochemical parameters, okra, etc.

INTRODUCTION

Nanotechnology, a new emerging and fascinating field of science, permits advanced research in many areas, and also opens up novel applications in the field of biotechnology and agriculture. The introduction of nanoparticles into plants might have a significant impact on better growth and yield of crops (Josko and Oleszczuk, 2013). Although fertilizers are important for plant growth and development, most of the applied fertilizers are rendered unavailable to plants due to many factors, such as leaching, degradation by photolysis, hydrolysis, and decomposition. Hence, it is necessary to minimize the losses in fertilization, and to increase the crop yield through the exploitation of new application techniques with the help of nanotechnology and nanomaterials. Nanoparticles (nano-scale particles = NSPs) are atomic or molecular aggregates with at least one dimension between 1 and 100nm (Ball 2002; Roco 2003), that can drastically modify their physico-chemical properties compared to the bulk material (Nel *et al.,* 2006).

The seeds of research in nanotechnology started growing for industrial applications nearly half a century ago, the drive for use of nanotechnology in agriculture came only recently with the reports published by Roco, the United States Department of Agriculture, the Nanoforum, and Kuzma and VerHage, along with similar publications. It has been envisioned that the novel properties of nanoscale biomaterials combined with creative engineering would have innovative applications for agriculture and food systems (Mukhopadhyay, 2014). In the recent years, multiple ways of interaction between the fields of nanotechnology and biology have been opened, with the development of tools for diagnosis and controlled delivery of substances. On the other hand, in the field of plant biology, the interaction between both disciplines has been less frequent. Most of the published work on this field has focus in the environmental impact of nanoparticles on crop growth and development; and also on the bio production of nanoparticles using plant extracts. (Corredor *et al.*, 2010).

Carbon nanotubes, graphene, known for their light weight, super strength and extreme conductivity, occupy a unique place in agricultural applications because of their abilities to affect seed germination, seedling growth, and plant development of various plant species. (Khodakovsk *et al.*, 2009).

Titanium dioxide NPs are promising as efficient nutrient source for plants to increase biomass production due to enhanced metabolic activities, and utilization of native nutrients by promoting microbial activities (Raliya *et al.*, 2013). TiO_2 increases metabolic activities and production of protein and carbohydrates which leads to enhance yield of the plant. (Chao *et al.*, 2007).

In view of the facts described above, an experiment was carried out to study the effect of graphene and Titanium dioxide nanoparticle on biochemical characteristics and yield of okra varieties.

MATERIALS AND METHODS:

The experiment was carried out during *zaid* season at Department of Biological Sciences, SHUATS, Allahabad, U.P. located at 25° 24' 42" N latitude, 81° 50' 56" E longitude and 98 m altitude above the mean sea level. The climate of Allahabad city is subtropical. The winter season is very cold with temperatures reaching as low as 2.5°C and summer season is very hot with temperatures reaching up to 48°C. The study was laid out in CRD design with six treatments and four replication. Seeds of two okra varieties (NAYAN-11&STAR-77) were used for the experimental purpose. Six different concentrations of nanoparticles were used as treatments *i.e.,* T0(control), T1(500 ppm graphene), T2(1000 ppm graphene), T3(25 ppm TiO_2), T4(50 ppm TiO_2), T5(100 ppm TiO_2).

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

1. Chlorophyll content (mg/g FW)

Chlorophyll content was determined according to Lichtenthaler, H. and Wellburn, A. (1983) 1 gram leaves sample was weighed and crushed with 80% acetone made the volume to 10 ml with 80% acetone, centrifuged at 800 ppm for 5 minutes. The supernatant was read under 663, 645 nanometre. The readings were fed in the following formula and results were determined under spectrophotometer.

Chlorophyll content 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. 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 is calculated by the formula –

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

3. Protein (%)

Protein was estimated by Lowry's method

Estimation of protein was done by pipetting out 50 µl supernatant containing proteins into test tubes in replicates of three and the total volume was made up to 1 ml. A tube with 1 ml distilled water served as a blank. 3 ml reagent C was added to each tube including the blank and after proper mixing the solution were allowed to stand for 30 min then 0.5 ml reagent D was added and after mixing, the tubes were left at room temperature in the dark for 60 min. Blue colour was developed in the solution. The absorbance was taken at 660 nm in UV-visible spectrophotometer. The amount of protein in the sample was calculated with the help of standard graph and expressed as mg/g leaf sample.

4. Carbohydrate (g): The total carbohydrate content was estimated by using the method given by Hedge and Hofreiter, 1962.

100mg of the leaf sample into a boiling tube, hydrolysed by keeping it in a boiling water bath for three hours with 5.0 ml of 2.5 N HCl and cooled to room temperature. Neutralized with solid sodium carbonate until the effervescence cease made the volume up to 100 ml and centrifuged, collected the supernatant and take 0.2 to 1.0 ml for analysis. Prepared the standards by taking 0.2-1.0 ml of the working standards. 1.0 ml of water serves as a blank made up the volume to 1.0 ml in all the tubes with distilled water, then added 4.0

ml of anthrone reagent, heated for eight minutes in a boiling water bath, cooled rapidly and read the green to dark green colour at 630 nm.

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.

5. 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.

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.

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 TiO2 (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.

The 100 ppm TiO_2 NPs noticeably promotes chlorophyll formation and stimulates Ribulose 1, 5-bisPhosphate carboxylase (Rubisco) activity and increases photosynthesis, thereby increasing plant growth and development. TiO_2 NPs protect chloroplast from aging for long time illumination (Yang *et al*., 2005). Similar observation was made by (Zheng *et al*., 2005) where the nano TiO_2 enhanced the photosynthetic carbon assimilation by activating (Rubisco) that could promote rubisco carboxylation, thereby increases the growth of plant.

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 100ppm 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).

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_2NPs regulates the enzyme activity involved in nitrogen metabolism such as nitrate reductase, glutamate dehydrogenase, glutamine synthase, and glutamic-pyruvic transaminase that helps the plants to absorb nitrate and also favours the conversion of inorganic nitrogen to organic nitrogen in the form of protein and chlorophyll that could increase the fresh weight and dry weight of plant (Mishra *et al*., 2014).

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 (2.35mg/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. Application of 100 ppm TiO_2 NPs on food crops has been reported to promote plant growth, increase the photosynthetic rate, reduce disease severity and enhance yield by 30%, as reported by Chao *et al*., (2007).

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.

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.

Table 1. Effect of graphene and titanium dioxide nanoparticles on biochemical characteristics in leaf tissue of okra varieties (NAYAN-11 and STAR-77)

	Chlorophyll-a (mg/g)		Chlorophyll-b (mg/g)		Total Chlorophyll (mg/g)		Carotenoids (mg/g)		Protein (%)		Carbohydrate (mg/g)	
Treatments	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

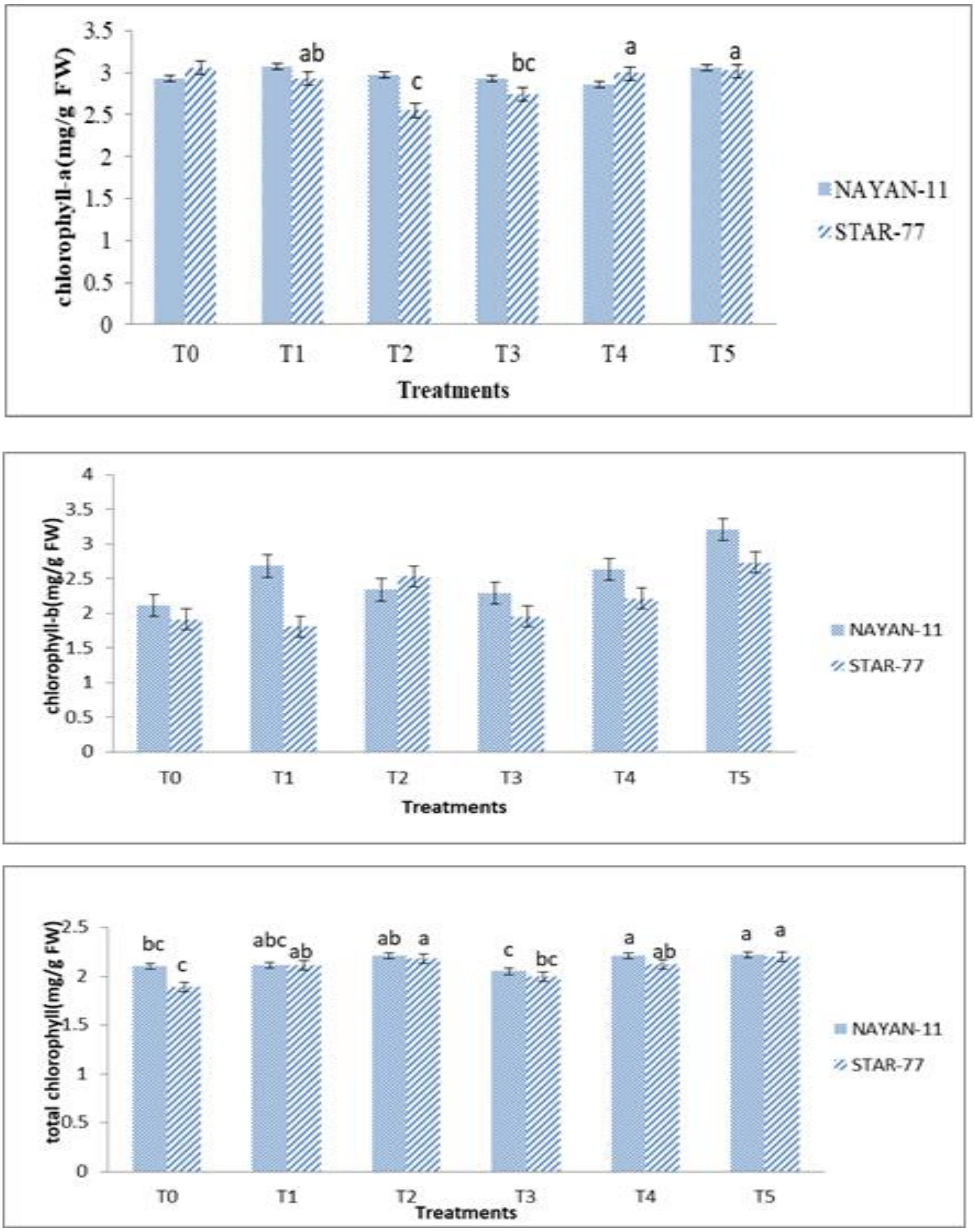

Fig 1. Effect of different concentration of graphene and titanium dioxide nanoparticles on chlorophyll-a, chlorophyll-b and total chlorophylls (mg/g) in leaf tissue of okra varieties (NAYAN-11 and STAR-77)

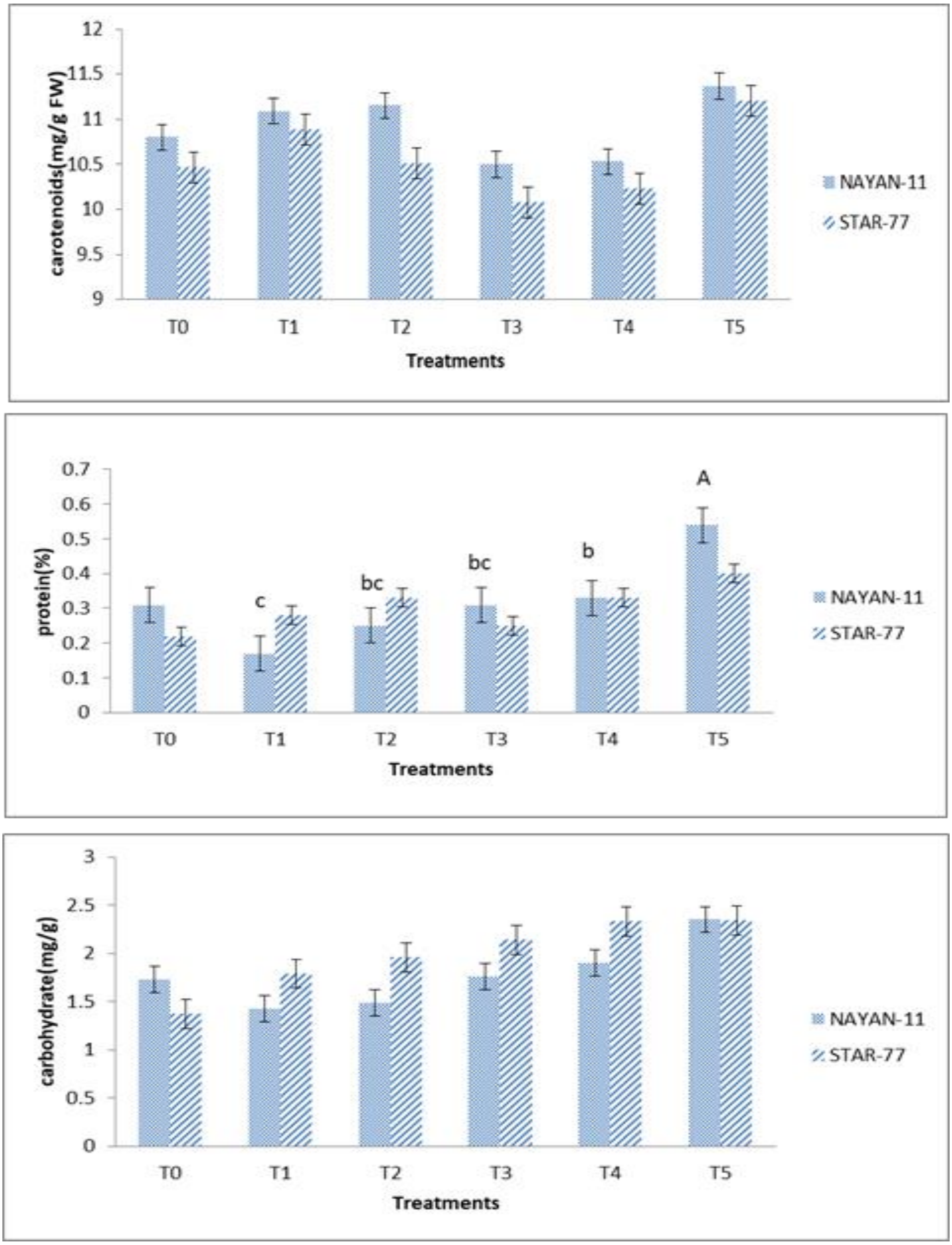

Fig 2. Effect of different concentration of graphene and titanium dioxide nanoparticles on carotenoids (mg/g), protein (%) and carbohydrates (g) in leaf tissue of okra varieties (NAYAN-11 and STAR-77)

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

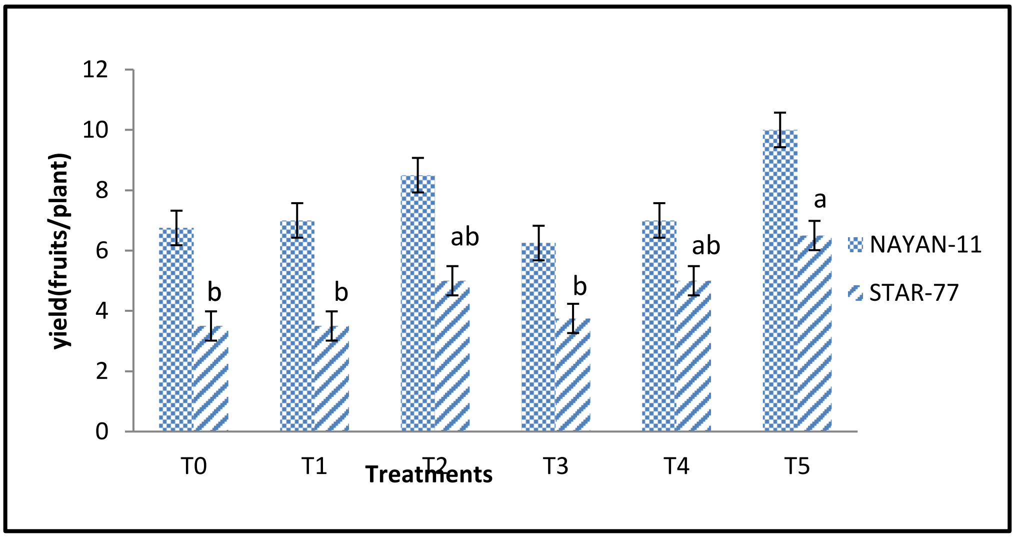

Fig 3. Effect of different concentration of graphene and titanium dioxide nanoparticles on yield (fruits/plant) in okra varieties.(NAYAN-11 and STAR-77)

Reference

Ball P. (2002). Natural strategies for the molecular engineer. Nanotechnology, 13: 15 – 28

Corredor, E., Risueno, M.C. and Testillano, P.S. (2010) Carbon-iron magnetic nanoparticles for Agronomic use in Plants. Plants Signalling and Behavior 5:10, 1295-1297.

Hedge, J.E. and Hofreiter, B.T. 1962. Carbohydrates chemistry. In: Whistler, R.L., Be Miller, J.B. 17, Academic Press, New York.

Hong, F., Yang, F., Liu, C., Gao, Q., Wan, Z., Gu, F., Wu, C., Ma, Z., Zhou, J. and Yang, P. (2005a). Influence of nano-TiO2 on the chloroplastaging of Spinach under light. *Biological Trace Element Research*, 104: 249 –260.

Josko and Oleszczuk,(2013). Influence of soil type and environmental conditions on ZnO, TiO2 and Ni nanoparticles phytotoxicity j.chemosphere.2013.02.048

Khodakovskaya M, Dervishi E, Mahmood M, Xu Y, Li Z, Watanabe F, Biris AS (2009) Carbon nanotubes m are able to penetrate plant seed coat and dramatically affect seed germination and plant growth (retracted article. see, vol. 6, pp. 7541, 2012). ACS Nano 3:3221–3227.

Lei, Z., Mingyu, S., Chao, .L., Liang, C., Hao, H., Xiao, W., Xiaoqing, L., Fan, Y., Fengqing, G. and Fashui, H. (2007) Effects of nanoanatase TiO2 on photosynthesis of spinach chloroplasts under different light illumination. *Biol Trace Elem Res* 119:68–76.

Lichtenthaler, H. and Wellburn, A. (1983) Determinations of Total Carotenoids and Chlorophylls a and b of Leaf Extracts in Different Solvents. Biochemical Society Transactions, 603, 591-592.

Mukhopadhyay, SS (2014). Nanotechnology in Agriculture: Prospects and Constraints. Invited review. Nanotechnology: Science and Applications 7, 63-71.

Mishra, V., Mishra, R.K., Dikshit, A. and Pandey, A.C. (2014) Interactions of nanoparticles with plants: an emerging prospective in the agriculture industry. *In: Ahmad P, Rasool S (eds) Emerging technologies and management of crop stress tolerance: biological techniques*, 1:159–180.

Nel, A., Xia, T., Madler, L. and Li, N. (2006). Toxic potential of materials at the nanolevel. *Science,* 311: 622 – 627.

Raliya, R., Tarafdar, J.C. (2013) ZnO nanoparticle biosynthesis and its effect on phosphorous-mobilizing enzyme secretion and gum contents in cluster bean (*Cyamopsis tetragonoloba* L.). Agricultural Research volume 2, pages48–57.

Zheng, L., Hong, F., Lu, S. and Liu, C. (2005) Effect of nano-TiO2 on strength of naturally aged seeds and growth of spinach. *Biol Trace Elem Res* 105:83–91.