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THE EFFECT OF TITANIUM DIOXIDE NANOPARTICLES AND SALICYLIC ACID ON GROWTH AND BIODIESEL PRODUCTION POTENTIAL OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) UNDER WATER STRESS

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Abstract

Biodiesel is a green fuel derived from vegetable oil and animal fats. In present studies we evaluated effects of salicylic acid (SA) and Titanium dioxide nanoparticles (nano-TiO_2) on growth and biodiesel production potential of sunflower (*Helianthus annuus* L.) varieties S-78 and Armoni under water deficit stress. The water deficit stress (50% and 30% field capacity of soil) was imposed during reproductive growth for one month. Salicylic acid (5 and 3 mg/L) and nano-TiO_2 (50 and 25 mg/L) was applied to leaves as a spray once before the start of water deficit stress period and next foliar spray 15 days after imposition of water stress. We observed that severe water stress (30% field capacity) significantly decreased plant leaf area, leaf relative water content (LRWC), chlorophyll *a, b* and carotenoids content, head diameter, width and length of achene, 1000 achene weight, achene oil content and biodiesel yield. Severe water stress (30% field capacity) increased oil acid value and free fatty acids content but decreased oil iodine value and refractive index. The foliar spray of salicylic acid and nano-TiO_2 effectively alleviated adverse effect of water deficiency stress on growth, achene quality and biodiesel yield of sunflower varieties. The sunflower variety Armoni performed better and showed better response to applied SA and Nano-TiO_2. Moreover, influence of nano-TiO_2 and SA on sunflower under water stress was nearly comparable. We concluded that foliar spray of either nano-TiO_2 (50 mg/L) or SA (5 mg/L) could be highly effective in the alleviation of adverse effect of water deficit stress on the biodiesel production potential of sunflower.

Key words: Titanium dioxide nanoparticles, Salicylic acid, Green fuel.

Introduction

Biodiesel is a green fuel synthesized from plant and animal based triglycerides. It is composed of alkyl esters of fatty acids (Ullah *et al*., 2013). For the production of biodiesel both edible (canola, safflower, soybean, sunflower) and none edible (*Jatropha curcus*, *Pongamia pinnata*) vegetable oil resources are used (Ullah *et al*., 2017). The production of biodiesel has been increased recently throughout the world. According to an estimate the world production of biodiesel was 284 Terawatt/hour in the year in 2018 (Nystrom *et al*., 2019). However, in developing countries like Pakistan commercialization of biodiesel is still a dream. The major reasons are that developing countries do not have sufficient amount of vegetable oil resources which can be exploited for large scale biodiesel production (Ullah *et al*., 2014). Moreover recent scenario of climate change in developing countries has caused severe reduction in the production of oil yielding crops (Ali *et al*., 2017). In such a situation, commercialization of biodiesel on a large scale can be achieved only by mitigating adverse effect of climate change on oil yielding plants and increasing their oil production capacity (Ullah, 2014).

Due to change in climate crops face environmental stresses like salinity, drought and temperature extremes (Latif *et al*., 2016; Shinwari *et al*., 1998, Nakashima *et al*., 2000). Among the various abiotic stresses water deficit stress is the most severe one and adversely effects all the developmental and growth phases of plants (Shinwari *et al*., 1998a; Narusaka *et al*., 1999; Sinclair, 2005; Salehi-Lisar *et al*., 2012). Oil yielding plants are more vulnerable to adverse effect of water stress (Ullah *et al*., 2013). A study showed that seed oil content of canola was decreased when exposed to water deficit stress during reproductive growth stage (Ullah *et al*., 2012). Similarly oil yield of sunflower was decreased (40%) due to water stress (Rauf, 2008). Water shortage during reproductive growth stages brought about changes in the concentration of major fatty acids in sunflower oil (Onemli, 2012). Studies of Ullah *et al*., (2013) concluded that alteration in weather conditions decreased oil and biodiesel yield of Sesame. In such a situation it is therefore required to take measures for the alleviation of adverse effect of water deficit stress on oil yielding plants which will assist in future commercialization of biodiesel without comprising on food products.

Nanotechnology is an emerging field of science in which particle size is reduced up to 10-100nm (Kasithevar *et al*., 2017). Nanoparticles have small size and large surface area which make them highly effective for chemical and biological applications (Rizvi & Saleh, 2018; Khalil *et al*., 2017). Titanium oxide nanoparticles exhibit optical and biological activities and have favorable effects on the growth and development of plants in trace amounts (Xie *et al*., 2011). Occurrence of TiO_2 has been observed in various environmental components like soil, water, plankton, fungi, aquatic animals and plants (Frazier *et al*., 2014). The Titanium dioxide nanoparticles increase the activity of antioxidant enzymes like Catalase, peroxidase, superoxidase dismutase and protect chloroplast form injurious effect of reactive oxygen species (Hong *et al*., 2005; Liu *et al*., 2021). Several experiments have shown that lower concentrations of titanium dioxide nanoparticles were not hazardous to living organisms (Zheng *et al*., 2012; Larue *et al.*, 2012). The TiO_2 nanoparticles stimulated growth of spinach plants by improving their rate of photosynthesis and nitrogen metabolism (Hong *et al.*, 2005; Yang *et al.*, 2008). Jaberzadeh *et al.*, (2013) reported that TiO_2 nanoparticles not only prevented losses in vegetative growth but also in yield of wheat due to water shortage.

Salicylic acid also known as ortho-hydroxy benzoic acid has beneficial effects on the physiology and biochemistry of many plant species. It improved growth of soybean and corn plants by modulating various physiological and biochemical pathways *(*Khan *et al.*, 2003; Shakirova, 2007*)*. Salicylic acid improved photosynthetic pigments content in leaves of maize (Khodary, 2004). Similarly, higher deposition of cell wall bound phenols occurred in leaves of maize upon application of salicylic acid which enhanced their tolerance to water deficit stress (Latif *et al.*, 2016). Salicylic acid applications significantly enhanced the dry matter content of *Brassica juncea* (Indian mustard) (Fariduddin *et al.*, 2003). It has a significant role in the uptake of ions, solute translocation, photosynthesis, induction of flowering, gene expression and endures stress resistance of plants (Wada *et al.*, 2010; Ullah *et al.*, 2012). It defends the leaf soluble proteins and retains normal leaf relative water content under drought stress (Khan *et al.*, 2012).

In present study we evaluated influence of nano-TiO_2 and salicylic acid on growth, oil yield and biodiesel production potential of sunflower varieties exposed to water stress.

Materials and Methods

Field experiments were carried out in University of Science and Technology Bannu during sunflower growing seasons of 2017 and 2018. Achenes of *Helianthus annus* Cvv. S-78 (Drought susceptible) and Armoni (Drought tolerant) were obtained from Agriculture Research Station Sari-e-Naurang Lakki Marwat, KP, Pakistan. The achenes were first washed with a 10% (v/v) aqueous solution of chlorox and then with distilled water. The achenes were sown in already prepared and weeds free field under natural environment conditions during spring of 2017 and 2018. The District Bannu lies at 32.9889°N, 70.6056°E Latitude. The experimental lay out was randomized complete block design, with four replica for each treatment. The soil of the field was a sandy loam containing 0.517% of organic matter (OM) with pH 7.57 and electrical conductivity (EC) of 0.506 ECum/cm. The soil contained 55% sand, 28% silt and 17% clay. Soil also contained inorganic matter CO_3 Meq/l (0.4%) and HCO_3 Meq/l (1.5%).

Solution of Salicylic acid (Sigma Chemicals Co. Ltd. USA) was prepared by dissolving requisite amount of hormone in 100 µL of ethanol and the volume was made to 0.1 L with distilled water. From this solution further 5mg/L and 3gm/L solutions were prepared.

The nanoparticles of TiO_2 were purchased from Sigma-Aldrich Inc. The particles size was <25 nm with 99.9% purity. The suspensions (50 and 25mg/L) of nano-TiO_2 were made in autoclaved distilled water using ultra-sonication method.

Plants were exposed to water deficient stress in the beginning of reproductive growth stage (80 days after water sowing). One set of plants was subjected to water deficiency stress for 30 days at 50% field capacity of soil (moderate water stress). Whereas the other set of plants was exposed to water stress for 30 days at 30% field capacity of soil (severe water stress).

One day before the onset of water stress condition plants were supplied with foliar spray of either SA or nano-TiO_2 using automated sprayer. Second spray of SA and nanoparticles was done 15 days after imposition of water stress. The treatment were: control plant sprayed with distilled water and not exposed to water stress, Water stress (50% field capacity), Water stress (30% field capacity), Water stress (50% field capacity) + SA (5 mg/L), Water stress level (50% field capacity) + SA (3 mg/L), Water stress level (50% field capacity) + nano-TiO_2 (50 mg/L), Water stress level (50% field capacity) + nano-TiO_2 (25 mg/L), Water stress (30% field capacity) + SA (5 mg/L), Water stress level (30% field capacity) + SA (3 mg/L), Water stress level (30% field capacity) + nano-TiO_2 (50 mg/L), Water stress level -2 + nano-TiO_2 (25 mg/L).

When period of water stress was completed, sampling was done on vegetative parts. Leaf width and length were determined by graph paper and leaf area was calculated. Leaf relative water content of 3rd healthy leaf from the shoot apex was determined. After excising fresh weight (FW) of leaf was determined followed by determination of its turgid weight (TW) and dry weight (DW) respectively.

LRWC (%) = [(weight of fresh leaf-weight of dry leaf) / (weight of turgid leaf-weight of dry leaf)] ×100

Leaf pigments like chlorophyll *a*, *b* and carotenoids were extracted and estimated using protocol of Arnon, (1949). Estimation of total soluble Phenolics content of leaves was done by Folin-Ciocalteau method (Adom & Liu, 2002).

All the plants in all sets were re-watered and permitted to grow to physiological maturity for yield and quality parameters determination. When plants reached to the stage of physiological maturity i.e., the backs of heads turned yellow and bracts became brown then they were harvested. The data were collected on diameter of head, width and length of achene and 1000 achene weight.

Oil was extracted from achenes in *n*-hexane using a Soxhlet extractor and achene oil content (%) was determined. Iodine value of oil was determined by using Anon., (1997) method Cd 1-25. The oil refractive index was determined by using a refractometer. The AOAC, (1984) method was adopted for determination of oil acid value. The achene oil (200 µg) was mixed with 2.5 ml diethyl ether: ethanol (1:1) and heated for complete dissolution. The mixture was later titrated with sodium hydroxide (0.1 N) by using phenolphthalein indicator. Free fatty acids content was derived from results of acid value as following:

$$\text{Acid value} = [56.1 \times N \times V] / W$$

Biodiesel was synthesized from achene oil of sunflower using standard protocol with slight modifications (Rashid *et al.*, 2008). The preheated oil (500 g) was treated with a mixture of sodium hydroxide (1 g) and methanol (40 ml) at 60 ± 1°C for three hours. The mixture was stirred continuously at 300 rpm for three hours and allowed to stand overnight for complete separation of glycerin and biodiesel phases. Biodiesel yield was expressed as % w/w conversion of sunflower oil into biodiesel.

Statistical analysis

Two way ANOVA was used for data analyses and treatment means comparison was done by least significant differences test (Statistix-8.1 USA).

Results

Both the moderate and severe stress decreased leaf area of Sunflower varieties then respective control ($p<0.05$). Lowest leaf area (13.8cm^2) was recorded under the treatment of severe stress. Both the treatments of SA and nano-TiO$_2$ minimized reduction in leaf area caused by water stress. Under moderate and severe stress beneficial effect of both the SA and nano-TiO$_2$ was similar. The two varieties of sunflower showed significant differences in leaf area. The cv Armoni had higher leaf area (31.806 cm$^{2)}$ then S-78 (30.159 cm^2). Under moderate stress (50% Field Capacity) Armoni was more responsive to applied nano-TiO$_{2.}$ Whereas, under severe stress both the varieties showed statistically equal response to foliar spray of SA and nano-TiO$_2$ (Table 1).

As compared with plants in control group those treated with moderate and severe water stress produced significantly smaller ($p<0.05$) head diameter (Table 1). However, smallest head diameter (10.23 cm) was recorded in plants treated with severe water stress (30% field capacity). Foliar application of SA and TiO$_2$ nanoparticles was effective and minimized reduction in head diameter both under moderate and severe water stress. Most effective doses of SA and nano-TiO$_2$ on head diameter under water stress were 5 mg/L and 50 mg/L. Effect of nano-TiO$_2$ on head diameter under water stress was statistically comparable to that of SA. Effect of varieties on head diameter was also significant ($p<0.05$). Significantly larger head diameter was recorded for cv S-78.

In our studies water stress had reducing effect on leaf relative water content (LRWC). Minimum value (49%) of LRWC was documented at 30% field capacity of soil (Table 2). All the treatments of SA and nano-TiO$_2$ showed positive effect on LWRC under water stress. However, SA was more effective when applied at 5mg/ L. Effectiveness of nano-TiO$_2$ on LRWC under water stress (30 % field capacity of Soil) was higher at 50 mg/L. The interaction between treatments and varieties was considerable. The Armoni was significantly more responsive to both the treatments of SA and nano-TiO$_{2.}$

Lowest chlorophyll (*a, b*) and carotenoids content was recorded in leaves of plants treated with severe water stress (30% field capacity) (Table 2). Foliar spray of SA and nano-TiO$_2$ lessened adverse effect of water stress on leaf chlorophyll and carotenoids content. It was noted that SA (5 mg /L) and TiO$_2$ nanoparticles (50 mg/L) were significantly more effective under water deficit stress. Interaction of treatments and varieties was also significant ($p<0.05$). Under severe water stress both the varieties showed almost equal response to applied SA and nano-TiO$_2$.

The total soluble phenolics content in the leaf was enhanced under low soil moisture availability (Fig. 1). Significantly upper level (19.08 mg Gallic acid equivalents/g f.w) of phenolics was observed in leaves of severe water stress treated plants. Foliar spray of both the SA and nano-TiO$_2$ further improved content of phenolics under stress. In state of both moderate and severe water stress influence of SA (5 mg/L and 3 mg/L) and nano-TiO$_2$ (50 mg/L and 25 mg/L) on leaf phenolics was similar. It was also noted that stimulatory outcome of SA and TiO$_2$ nanoparticles on leaf phenolics was much higher under severe water stress than that of moderate water stress. Both the varieties had similar content of phenolics in leaves. The Armoni was found as more responsive to the treatments of SA and nano-TiO$_2$ under moderate and severe water stress.

Both levels of water stress have reducing effect on achene length and width than control (Table 3). Compared with water stress treated plants, plants supplemented with foliar SA and nano-TiO$_2$ had better achene quality. Data revealed that impact of nano-TiO$_2$ on achene length and width under water stress was comparable to SA irrespective of the intensity of water stress. Under severe water stress (30% field capacity) cv Armoni was more responsive to nano-TiO$_2$ (50 mg/L) then cv S-78.

Table 1. Nano-TiO$_2$ and SA effect on morphological parameters of sunflower under water deficit stress.

Treatments	Leaf area (cm^2)		Head diameter (cm)	
	S-78	Armoni	S-78	Armoni
Control	43.759 B ± 1.337	52.502 A ± 0.979	11.667 A ±0.12	11.667 A ± 0.088
Water stress (50% FC)	16.200 JK ± 1.179	17.234 J ± 0.597	11.033 C-F ± 0.088	10.333 HI ± 0.12
Water stress (30% FC)	12.479 L ± 0.489	13.786 KL ± 1.027	10.467 GH ± 0.176	10.0 J ± 0.057735
Water stress (50% FC)+SA (5mg/L)	34.556 EF ± 0.578	36.948 C-E ± 1.308	11.133 B-E ± 0.185	11.133 B-E ± 0.203
Water stress (50% FC)+ TiO$_2$ (50mg/L)	35.147 EF ± 0.536	38.144 C ± 0.744	11.333 BC ± 0.088	11.367 AB ± 0.088
Water stress (50% FC) + SA (3mg/L)	33.22 FG ± 0.499	35.615 D-F ± 0.318	10.900 EF ± 0.0578	10.467 GH ± 0.033
Water stress (50% FC)+ TiO$_2$ (25mg/L)	34.447 F ± 0.2478	37.802 CD ± 0.871	11.267 B-D ± 0.120	10.767 FG ± 0.145
Water stress (30%FC)+SA (5mg/L)	30.042 HI ± 1.316	29.332 HI ± 0.684	11.067 B-F ± 0.12	11.067 B-F ± 0.089
Water stress (30%FC)+TiO$_2$ (50mg/L)	31.115 GH ± 1.197	30.156 HI ± 0.747	11.033 C-F ± 0.057	11.0 D-F ± 0.089
Water stress (30%FC)+SA (3mg/L)	29.709 HI ± 0.488	28.666 I ± 0.487	10.267 H-J ± 0.088	10.1 IJ ± 0.057
Water stress (30% FC) + TiO$_2$ (25mg/L)	31.066 G-I ± 0.928	29.686 HI ± 0.682	11.0 D-F ± 0.058	10.267 H-J ± 0.120
Mean	**30.159 B**	**31.806 A**	**11.012 A**	**10.745 B**

Table 2. Nano-TiO_2 and SA effect on physiological parameters of sunflower under water deficit stress.

Treatments	Leaf relative water content (%)		Chlorophyll *a* content (mg/g f.w)		Chlorophyll *b* content (mg/g f.w)		Carotenoids content (mg/g f.w)	
	S-78	Armoni	S-78	Armoni	S-78	Armoni	S-78	Armoni
Control	55.474H ± 0.577	57.847 FG ± 0.285	6.927 A ± 0.036	6.943 A ± 0.102	2.032 CD ± 0.037	1.972C-G ± 0.024	1.134 C-F ± 0.058	1.195 CD ± 0.098
Water stress (50% FC)	51.186 I ± 0.895	56.678 GH ± 0.156	4.272 J ± 0.141	4.437 J ± 0.017	1.986 C-F ± 0.124	4.437 J ± 0.0614	0.949 FG ± 0.025	0.632 H ± 0.177
Water stress (30% FC)	43.314 J ± 0.559	55.103 H ± 1.615	3.241K ± 0.098	3.336 K ± 0.069	2.756 A ± 0.056	1.913 D-I ± 0.056	0.493 H ± 0.098	0.204 I ± 0.07
Water stress (50% FC)+SA (5mg/L)	62.123 D ± 0.528	65.779 C ± 0.035	6.121 DE ± 0.071	6.287 CD ± 0.158	1.952 C-H ± 0.0673	1.703 J-L ± 0.096	1.031 D-G ± 0.015	1.92 A ± 0.0672
Water stress (50% FC)+ TiO_2 (50mg/L)	57.634 FG ± 1.047	70.723 A ± 1.266	6.454 BC ± 0.071	6.554 B ± 0.043	1.878 D-J ± 0.044	1.666 KL ± 0.036	1.168C-E ± 0.035	1.95 A ± 0.034
Water stress (50% FC) + SA (3mg/L)	60.789 DE ± 0.394	65.461 C ± 0.237	5.979 EF ± 0.071	6.021 E ± 0.081	1.907 D-J ± 0.018	1.754 H-L ± 0.009	0.93 G ± 0.042	1.554 B ± 0.035
Water stress (50% FC)+ TiO_2 (25mg/L)	56.834 GH ± 0.869	70.603 A ± 1.217	6.254 CD ± 0.071	6.004 E ± 0.002	1.995 C-F ± 0.067	1.997 C-E ± 0.001	1.068 D-G ± 0.035	1.087 D-G ± 0.007
Water stress (30%FC)+SA (5mg/L)	65.089 C ± 0.0271	65.935 C ± 0.0516	5.295 H ± 0.085	5.195 HI ± 0.015	1.816 E-K ± 0.099	1.722 I-L ± 0.099	1.044 D-G ± 0.069	1.893 A ± 0.052
Water stress (30%FC)+TiO_2 (50mg/L)	56.991 GH ± 0.478	68.500 B ± 0.434	5.761 FG ± 0.071	5.562 G ± 0.031	2.046 CD ± 0.171	1.588 L ± 0.158	1.089 D-G ± 0.049	1.9 A ± 0.053
Water stress (30%FC)+SA (3mg/L)	59.689 EF ± 0.675	65.681 C ± 0.183	5.162 HI ± 0.044	5.025 I ± 0.122	1.771 G-L ± 0.032	1.791 F-L ± 0.015	0.666 H ± 0.089	1.293 C ± 0.059
Water stress (30% FC) + TiO_2 (25mg/L)	56.625 GH ± 0.659	68.862 AB ± 0.854	5.0017 I ± 0.1297	5.002 I ± 0.009	2.476 B ± 0.099	1.665 KL ± 0.0007	0.989 E-G ± 0.049	1.017 D-G ± 0.001
Mean	**56.886 B**	**64.652 A**	**5.5602 A**	**5.4878 B**	**2.056 A**	**1.808 B**	**0.9603 B**	**1.3318 A**

Table 3. Nano-TiO_2 and SA effect on achene quality of sunflower under water deficit stress.

Treatments	Achene length (mm)		Achene width (mm)		Weight of 1000 Achenes (g)		Achene oil content (%)	
	S-78	Armoni	S-78	Armoni	S-78	Armoni	S-78	Armoni
Control	12.400 A ± 0.05	12.267 A ± 0.1453	6.9 A ± 0.058	6.667 AB ± 0.145	7.013 A ± 0.009	7.033 A ± 0.009	39.467A ± 0.03	40.887A ± 0.04
Water stress (50% FC)	6.167 CD ± 0.088	6.4 CD ± 0.058	5.533 H ± 0.146	5.5667 H ± 0.145	4.22 H ± 0.006	4.33 H ± 0.067	30.333 M ± 0.06	31.343L ± 0.098
Water stress (30% FC)	5.233 D ± 0.145	5.300 D ± 0.1	5.167 I ± 0.120	4.933I ± 0.088	4.027 I ± 0.006	4.01 I ± 0.012	29.537 M ± 0.03	30.343 ± 0.01m
Water stress (50% FC)+SA (5mg/L)	11.100 AB ± 0.058	11.233 AB ± 0.088	6.633 AB ± 0.145	6.6 A-C ± 0.153	6.477 B ± 0.016	6.523 B ± 0.015	39.000 DE ± 0.02	41.053 A ± 0.03
Water stress (50% FC)+ TiO_2 (50mg/L)	11.500 AB ± 0.115	11.467 AB ± 0.033	6.8 A ± 0.057735	6.667 AB ± 0.12	6.594 B ± 0.043	6.597 B ± 0.031	36.817 F ± 0.3	39.387 CD ± 0.02
Water stress (50% FC) + SA (3mg/L)	11.067 AB ± 0.088	10.933 AB ± 0.033	6.267 DE ± 0.120	6.267 DE ± 0.120	6.143 D-F ± 0.0233	6.34 C ± 0.066	38.300 E ± 0.41	40.887 A ± 0.01
Water stress (50% FC)+ TiO_2 (25mg/L)	11.200 AB ± 0.058	10.800 AB ± 0.115	6.467B-D ± 0.033	6.4 B-E ± 0.0577	6.223C-E ± 0.049	6.2300 C-E ± 0.035	36.383 FG ± 0.11	38.973 DE ± 0.04
Water stress (30%FC)+SA (5mg/L)	11.100 AB ± 0.058	11.200 AB ± 0.058	6.3 C-E ± 0.115	6.1667D-F ± 0.120	6.1233 EF ± 0.008	6.567 B ± 0.0167	36.800 F ± 0.03	39.993 BC ± 0.31
Water stress (30%FC)+TiO_2 (50mg/L)	8.003 C ± 3.397	11.333 AB ± 0.033	6.4 B-E ± 0.111	6.267 DE ± 0.088	6.26 CD ± 0.049	6.2867 C ± 0.068	35.467 HI ± 0.03	40.327 AB ± 0.01
Water stress (30%FC)+SA (3mg/L)	10.7 AB ± 0.057	10.100 B ± 0.058	5.9 FG ± 0.0577	5.9 FG ± 0.057	6.053 F ± 0.024	6.12 EF ± 0.035	35.800 GH ± 0.351	39.793 B-D ± 0.273
Water stress (30% FC) + TiO_2 (25mg/L)	11.033 AB ± 0.033	10.467 AB ± 0.120	6.133 EF ± 0.033	5.767 GH ± 0.088	6.123 EF ± 0.008	5.8 G± 0.153	34.847 IJ ±0.17	39.327 ± 0.01cd
Mean	**9.955 A**	**10.136 A**	**6.2273 A**	**6.1091 B**	**5.9309 B**	**5.9867 A**	**0.8336 B**	**37.815 A**

Table 4. Nano-TiO_2 and SA effect on oil quality indices of sunflower under water deficit stress.

Treatments	Iodine Value of oil (g I/100g oil)		Oil acid value (mg KOH/g oil)	
	S-78	Armoni	S-78	Armoni
Control	105.33 a ± 0.08	105.00 a-c ±0.05	0.67 k ± 0.006	0.593l ± 0.01
Water stress (50% FC)	102.18 j ± 0.16	103.13 i± 0.08	0.873 FG ± 0.09	0.963BC ± 0.09
Water stress (30% FC)	101.27 k ± 0.145	102.20 j ± 0.08	0.963BC ± 0.09	1.027A ± 0.01
Water stress (50% FC)+SA (5mg/L)	104.57 de ± 0.05	104.70b-d ± 0.1	0.783 J ± 0.01	0.863GH ± 0.09
Water stress (50% FC)+ TiO_2 (50mg/L)	105.33 A ± 0.01	105.03AB ± 0.08	0.773 J ± 0.09	0.873 FG ± 0.01
Water stress (50% FC) + SA (3mg/L)	104.30EF ± 0.05	104.17FG ± 0.09	0.867GH ± 0.09	0.9EF ±0.01
Water stress (50% FC)+ TiO_2 (25mg/L)	105.0 A-C ± 0.08	104.67CD ± 0.09	0.837HI ± 0.09	0.97 BC ± 0.05
Water stress (30%FC)+SA (5mg/L)	104.73 B-D ± 0.01	104.9 B-D ± 0.09	0.8633GH ±0.02	0.94D ± 0.05
Water stress (30%FC)+TiO_2 (50mg/L)	104.30 EF± 0.05	104.13FG ± 0.25	0.8633 GH ± 0.07	0.98 B ± 0.06
Water stress (30%FC)+SA (3mg/L)	103.87GH ± 0.08	104.0FG ± 0.05	0.8567 GH ± 0.01	0.91E ± 0.05
Water stress (30% FC) + TiO_2 (25mg/L)	103.63 H ± 0.07	104.03FG ± 0.08	0.82GH ± 0.06	0.92DE ± 0.05
Mean	**104.05 B**	**104.18 A**	**0.8336 B**	**0.9042 A**

Both the water stress levels drastically decreased achene weight than control (Table 3). Maximum decrease in achene weight was observed under sever water stress. Compared with group of plants exposed to water stress levels, plants applied with foliar spray of SA and nano-TiO_2 had higher achene weight after water stress. In condition of moderate water stress beneficial effect of nano-TiO_2 on achene weight was statistically similar to SA. It was noted that most effective doses of SA and nano-TiO_2 were 5 mg/L and 50 mg/L respectively (Table 3). Two way ANOVA showed that interaction of treatments and varieties for achene weight was significant. Under moderate and sever water stress response of cv Armoni was higher to foliar spray of both the concentrations of SA. Whereas under severe water stress beneficial effect of nano-TiO_2 (25 mg/L) was higher than SA on achenes weight of cv Armoni.

Achene oil content was decreased under both the levels of water stress (50% and 30% Field Capacity) then non stressed control (Table 3). Lowest oil content (33.92 %) was recorded in achenes of plants treated with severe water stress (30% FC). Under moderate stress (50% FC) foliar application of SA at both concentrations (5 mg/L and 3 mg/L) minimized water stress effect and retained normal achene oil content after water stress. Like SA nano-TiO_2 lessened decreasing effect of water stress on oil content. However, under moderate water stress SA was more effective than nano-TiO_2. Under severe water stress foliar spray of SA (5 mg/L) and nano-TiO_2 (50 mg/L) was equally effective in the alleviation of water stress effect on oil content. However, at same level of water stress (30% FC) SA (3 mg/L) was more effective on oil content than nano-TiO_2. Interaction between variety and treatment for oil percent was significant ($p<0.05$). Water stress decreased oil content of both the varieties. However, foliar application of SA and nano-TiO_2 gave highly encouraging result in Armoni under moderate and severe water stress than S-78. Data further revealed that Armoni had higher oil content then S-78.

Lowest iodine value (101.735 g I_2 /100g of oil) was found in achene oil of plants treated with sever water stress (30 % FC) which was 105.15 g I2 /100g of oil for control (Table 4). Under moderate water stress (50 % FC) nano-TiO_2 (50mg/L) maintained normal iodine value of oil. However under severe water stress SA (5mg/L) was more effective than both doses of nano-TiO_2. Interaction of treatments and varieties for iodine value of oil was significant. Both under moderate and severe water stress higher reduction in iodine value were found in achene oil of cv S-78 than cv Armoni. Data showed that response of both the varieties to all treatments of SA and nano-TiO_2 was nearly similar. Data also showed that iodine value was higher in achene oil of Armoni than S-78.

Both the levels of water stress increased acid value of oil (Table 4). In condition of moderate water stress, the SA (5mg/L) and nano-TiO_2 (50 mg/L) decreased the oil acid value. The decreasing effect of nano-TiO_2 (50 mg/L) was statistically comparable to that of SA (5 mg/L). Similarly the increase in oil acid value caused by severe water stress was significantly minimized by SA (5 mg/L) and nano-TiO_2 (50 mg/L). However application of SA and nano-TiO_2 foliar spray had similar effect on both varieties S-78 and Armoni.

Both the levels of water stress increased the content of free fatty acids in achene oil (Fig. 2). Maximum free fatty acids were recorded in oil of plants exposed to severe water stress. In condition of moderate water stress, the SA (5 mg/L) and nano-TiO_2 (50 mg/L) decreased the content of free fatty acids. The decreasing effect of nano-TiO_2 (50 mg/L) was statistically comparable to that of SA (5 mg/L). Similarly the increase in free fatty acids caused by severe water stress was significantly minimized by SA (5 mg/L) and nano-TiO_2 (50 mg/L).

Our results showed that both the levels of water stresses (50% and 30% field capacity) decreased (95.33 and 89.5%) biodiesel yield of sunflower oil respectively (Fig. 3). The results confirmed that at moderate water stress (50% field capacity) application of nano-TiO_2 (50 mg/L) and salicylic acid (5 mg/L) significantly reduced water stress effect and increased biodiesel yield of both the varieties of sunflower.

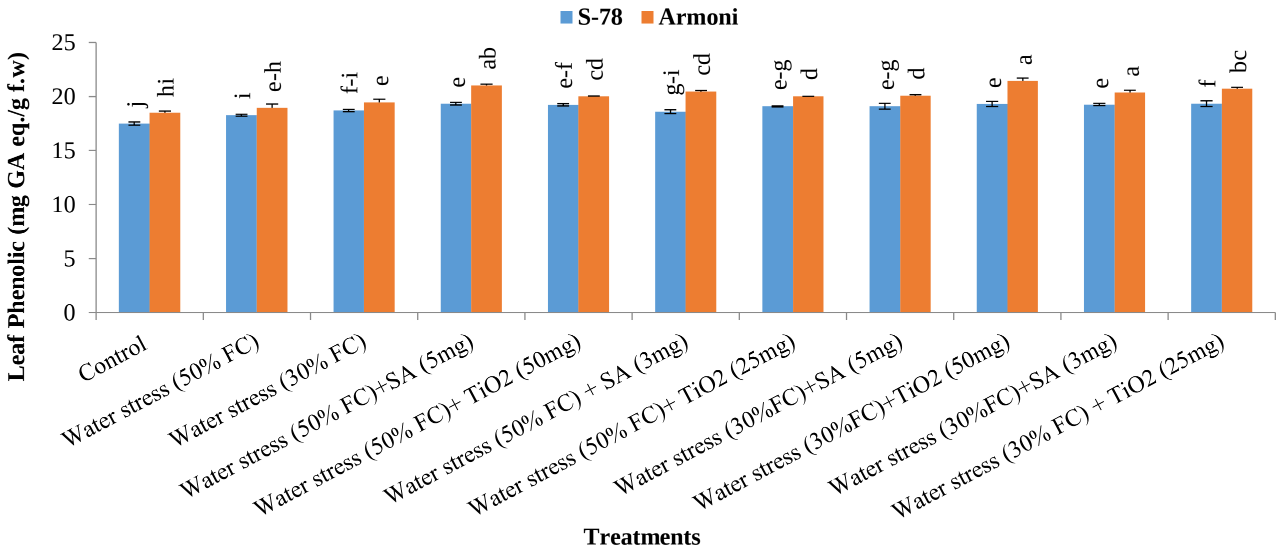

Fig. 1. Nano-TiO_2 and SA effect on Leaf Phenolics of sunflower under water deficit stress.

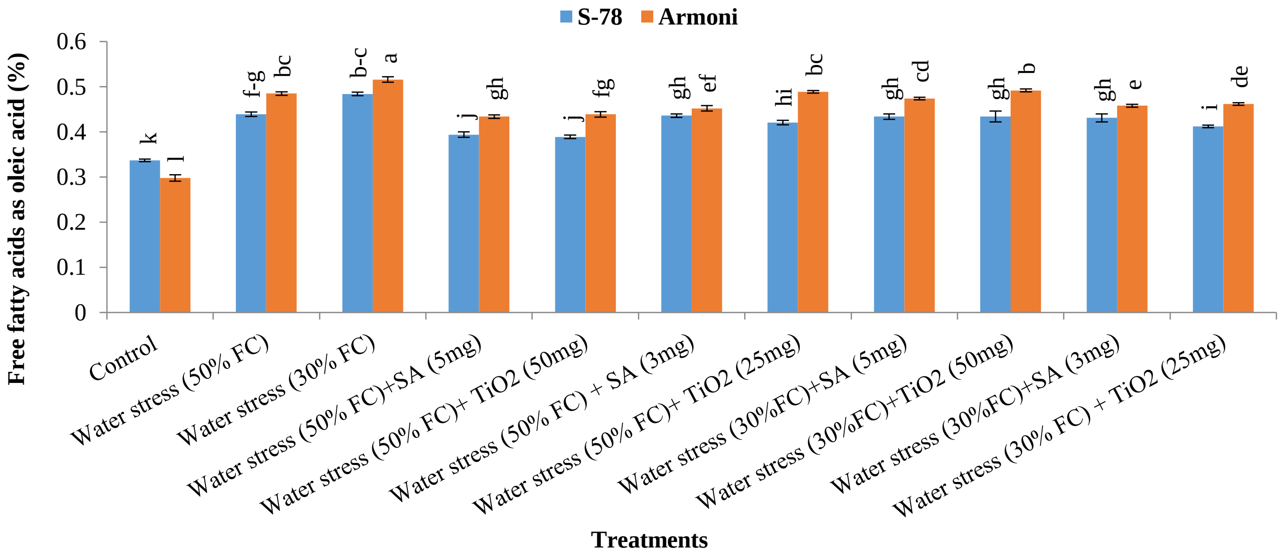

Fig. 2. Nano-TiO_2 and SA effect on free fatty acid value of sunflower under water deficit stress.

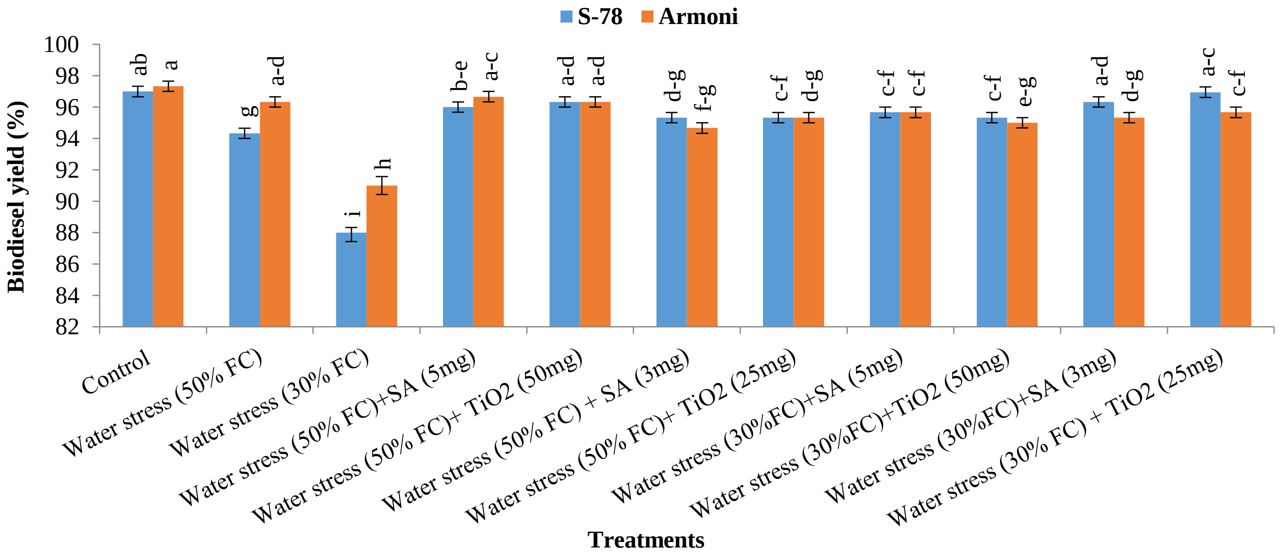

Fig. 3. Nano-TiO_2 and SA effect on Biodiesel yield of sunflower under water deficit stress.

Discussion

Reduction in leaf area results due to decrease in leaf size (Toscano *et al*., 2014). Water deficiency stress causes inhibition of cell division and limits leaf enlargement (Jaleel *et al*., 2008). Water deficit stress adversely affected leaf area of both the varieties of sunflower. Foliar spray of both SA and Nano-TiO$_2$ sustained leaf area of sunflower varieties under water stress. Both the SA and Nano-TiO$_2$ had comparatively similar effect on the leaf area. Leaf area reduction is a common plant response to water shortage (Toscano *et al*., 2018). The SA has stimulatory effect on mitotic activity in growth apices of plant (Pasternak *et al*., 2019) and thus modifies leaf area under condition of water stress (Cornnelia *et al*., 2010). Studies of Abdul-Latef *et al*., (2018) showed that Nano-TiO$_2$ protected leaf area of broad bean from injurious effect of salt stress. The beneficial effect of TiO$_2$ nanoparticles on leaf area in water deficit stress can be supported by the fact that many abiotic stresses related genes are up regulated by Nano-TiO$_2$ treatment (Tumbura *et al*., 2017). The TiO$_2$ nanoparticles influence growth characteristics of plants in a dose dependent manner (Yaqoob *et al*., 2017). The Nano-TiO$_2$ was found to counter act water deficient stress in wheat by improving agronomical parameters (Jaberzadeh *et al*., 2013).

Water stress decreased achene length, width and weight. Seed filling involves the transport and mobilization of various constituents and complex biochemical processes for the synthesis of lipids, proteins and carbohydrates in the developing seeds (Farooq *et al*., 2017; Sehgal *et al*., 2018). Water deficiency stresses inhibits activities of enzymes involved in the synthesis of storage material of seeds (Ahmadi & Baker 2001). In our studies foliar spray of SA and nano-TiO$_2$ lessened water stress effect on achene length, width and weight. The SA has been reported to increase translocation of photo assimilates to developing seeds (Ullah *et al*., 2012). Moreover, SA has a role in maintaining turgidity of cells which is necessary for normal division and expansion of cotyledonary cells in developing seeds/grains (Sehgal *et al*., 2018). The nano-TiO$_2$ effect on the quality parameters of sunflower achene was indirect which might be due to its beneficial effects on the protection of chloroplast from oxidative stress by improving content of phenolics in leaves during water stress (Aghdam *et al*., 2016). Azmat *et al*., (2020) reported increase in growth indexes of *Spinacia oleracea* due to nano-TiO$_2$ treatment by improving their photosynthetic efficacy. The nano-TiO$_2$ promotes light absorption capacity of chloroplast by up regulating genes related to light harvesting complex II (Ze *et al*., 2011). As reported by Hale *et al*., (2005) hiher phenolic production improved tolerance of plants to soil moisture deficiency stress. Phenolic compounds are used by plant cells to stabilize all form of reactive oxygen species. Moreover, these phenolics compounds increase cell wall thickness when deposited under water stress ensuring lower flow of water from inside of cell (Hura *et al*., 2013; Latif *et al*., 2016). Latif *et al*., (2016) studies showed stimulatory effect of SA on the biosynthesis of endogenous phenolics in maize leaves. Higher synthesis of phenolics under water stress occurs at the expense of photosynthesis outcome resulting in decrease dry mass production (Hura *et al*., 2017). Both SA and nano-TiO$_2$ not only improved content of leaf phenolics but also achene quality under water stress.

Water stress decreased seed oil content of sunflower varieties. Seed oil content is very important in perspectives of oil yield. It was demonstrated in a study that water deficiency stress changed oil protein ratio of canola varieties (Ullah *et al*., 2012). The SA and Nano-TiO$_2$ positively affected achene oil content of sunflower varieties under water stress. These beneficial effects of SA and Nano-TiO$_2$ could be attributed to their positive effects on physiological and biochemical considerations of sunflower plants.

Water deficiency stress increased oil acid value and free fatty acid content which resulted in a lower biodiesel yield. However, SA and Nano-TiO$_2$ prevented increase in these quality measuring parameters of sunflower oil. Presence of higher content of free fatty acid in vegetable oils results in a lower ratio of fatty acid conversion into their respective methyl ester (Ullah *et al*., 2014). Free fatty acids are converted into soap rather than alkyl esters in an alkali canalized transesterification reaction and results in lower biodiesel yield (Ullah *et al*., 2017). In our studies the SA and TiO$_2$ nanoparticles application prevented increase in the content of free fatty acids in oil of achenes. That's why the oil obtained from plants treated with SA and Nano-TiO$_2$ under water stress gave higher yield of biodiesel than untreated ones. Additional researches to define influence of SA and nano-TiO$_2$ on enzymes involved in the synthesis of fatty acid need to be explored. Our study established that foliar spray of SA and nano-TiO$_2$ improved biodiesel yield of sunflower directly by improving oil content and indirectly by decreasing oil free fatty acid content under water stress. Studies showed that application of nano-TiO2 on oilseed plants helped in formation of polydentate sulfate species inside the structure of TiO$_2$ while enhanced the stability of synthesized TiO$_2$ nano catalyst and present a higher tolerance to free fatty acids in raw material for biodiesel production (Carlucci *et al*., 2019).

Conclusions

Severe water deficit stress (30% Field capacity) badly affected the growth, yield, oil quality and biodiesel production potential of sunflower. The foliar spray of SA and nano-TiO$_2$ minimized water stress effect on leaf area, leaf relative water content, head diameter, achene oil quality and biodiesel yield. Moreover, we noted that foliar spray of both the SA and nano-TiO$_2$ enhanced the buildup of phenolics in leaves under water stress which may be a mechanism behind water stress resistance of sunflower varieties. The foliar application of nano-TiO$_2$ (50 mg/L) and SA (5 mg/L) is recommended for lessening water stress effects on biodiesel production potential of sunflower.

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