Electronic Supplementary Information (ESI) for:

Cryo-milled nano-DAP for enhanced growth of monocot and dicot plants

Naorem Ronald Reagan Singh,[a] Sreedhara Sudhakara Sarma,[b] Tata Narsinga Rao,[b] Harita Pant,[c] Vadali Venkata Satya Siva Srikanth[c] and Rahul Kumar*[a]

[a] Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Gachibowli, Hyderabad – 500046, Telangana, India.

[b] International Advanced Research Centre for Powder Metallurgy and New Materials (ARCI), Hyderabad –500005, Telangana, India.

[c] School of Engineering Sciences and Technology, University of Hyderabad, Gachibowli, Hyderabad – 500046, Telangana, India.

*Corresponding author: Rahul Kumar

E-mail: rksl@uohyd.ac.in

Germination of wheat and tomato seeds. Tomato seeds were taken in a 50 ml Falcon tube and mixed with 25 ml of 4% NaOCl, capped and vortexed at high speed for 8 min. The seeds are then rinsed with doubled distilled water (dd H_2O) until the NaOCl is completely washed off. In the case of wheat seeds NaOCl was not used because it hampers the germination efficiency. Instead, the process of seed wash utilized dd H_2O, vortexed until the water was devoid of free-floating debris. In both the cases the seeds were germinated on a dd H_2O moistened blotting paper under skotomorphogeneic conditions.

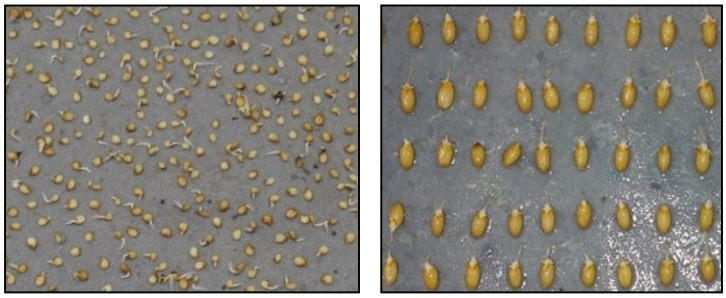

Fig. S1 Germinated tomato (left) and wheat seedlings (right).

Manually prepared modified ½ X MS media. The composition and strength of the media utilized in this study are given in Table S1. The media need not be autoclaved as there is no organic acid and amino acid in the manually prepared modified ½X MS media preparation. Double distilled water is used as solvent.

Table S1. Composition for the manual preparation of modified ½X MS media.

Sl. No.	Chemical	Amount (mg/L)
1	KH_2PO_4*	170.000
2	$MgSO_4.7H_2O$	90.345
3	NH_4NO_3	825.000
4	$CaCl_2.2H_2O$	166.100
5	KCl	70.050
6	H_3BO_3	3.100
7	$MnSO_4.H_2O$	8.450
8	$ZnSO_4.7H_2O$	4.300
9	$Na_2MoO_4.2H_2O$	0.1065
10	$CuSO_4.5H_2O$	0.0125
11	$CoCl_2.6H_2O$	0.0125
12	$FeSO_4.7H_2O$	13.900
13	$Na_2EDTA.2H_2O$	18.650
14	KI	0.415

* KH_2PO_4 is removed in case of P free manual prepared modified ½X MS media.

Solid Growth Media. Solid growth media is prepared by uniformly mixing 30 g of commercial perlite (Divine Tree®, India) with 300 g of locally available non-fertilized sand (with a Pi content of 0.646 nM/mg) moistened with 150 ml of manually prepared P-free modified ½X MS media. The diameter for the mouth opening of the pot used for growing seedlings with solid growth media is 11 cm, has a height of 9.5 cm and a base diameter of 7.9 cm. The base is invaginated inwards which accommodates holes.

Table S2**.** Details of the experimental setup for n-DAP efficiency test when the seedlings are grown using a solid media of sand and perlite mixture.

Pot. No.	SAMPLE	Dosage per Pot (mg)	Sand (g)/Pot	Perlite (g)/Pot	
1	Positive Control (KH_2PO_4)	68.255	300	30	1) Moistened with 150 ml of manually prepared P-free modified ½X MS media. 2) Supplement 50 ml of manually prepared P-free modified ½X MS media after every 3 days to maintain consistent moisture.
2	Negative Control (0 P)	0	300	30	
3	10% n-DAP	6.825	300	30	
4	25% n-DAP	17.063	300	30	
5	50% n-DAP	34.127	300	30	
6	100% n-DAP	68.255	300	30	
7	10% c-DAP	6.825	300	30	
8	25% c-DAP	17.063	300	30	
9	50% c-DAP	34.127	300	30	
10	100% c-DAP	68.255	300	30	

In case of the pot used for hydroponics, the mouth opening has a diameter of 13.8 cm, a height of 11.3 cm and a base diameter of 9.8 cm.

Table S3**.** Details of the experimental setup for n-DAP efficiency test when the seedlings are grown hydroponically.

Pot. No.	SAMPLE	Dosage per Pot of 1 L (mg)	Media Mixture/Pot
1	Positive Control (KH_2PO_4)	27.218	1) 1 L of manually prepared P-Free modified ½X MS media 2) Manually prepared P-free modified ½X MS media supplemented every 3 days to maintain at 1 L mark 3) Clay balls were placed on top of the media to prevent algal growth and reduce evaporation.
2	Negative Control (0 P)	0	
3	25 µM n-DAP	3.301	
4	50 µM n-DAP	6.603	
5	100 µM n-DAP	13.206	
6	200 µM n-DAP	26.412	
7	25 µM c-DAP	3.301	
8	50 µM c-DAP	6.603	
9	100 µM c-DAP	13.206	
10	200 µM c-DAP	26.412	

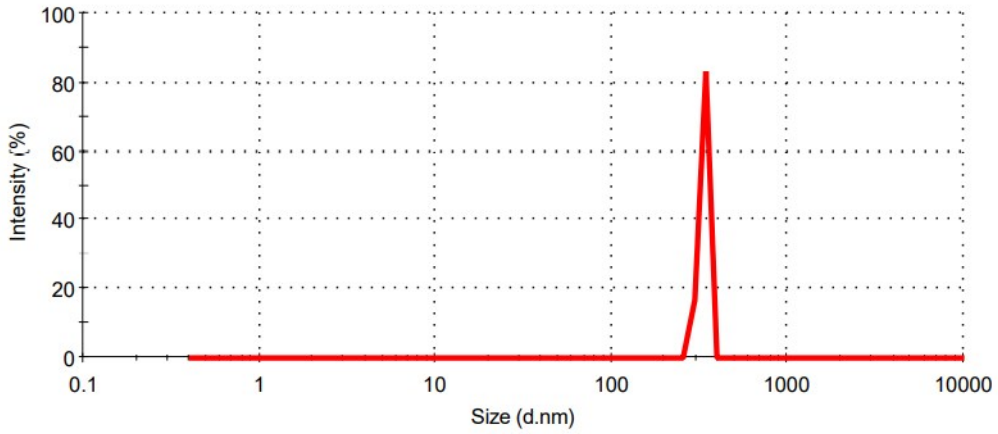

Fig. S2 Particle size distribution by intensity (DLS) of n-DAP particles.

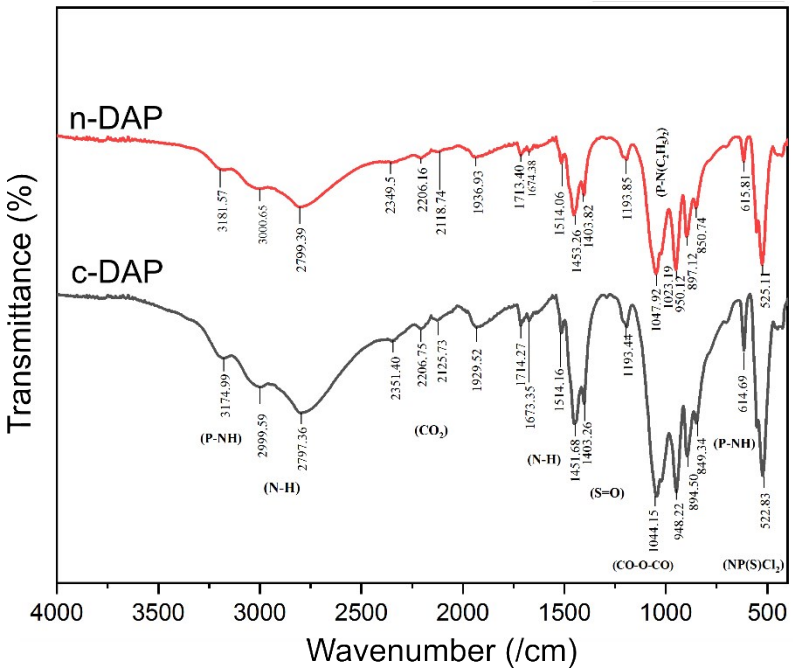

Fig. S3 FTIR spectra of c-DAP and n-DAP particles.

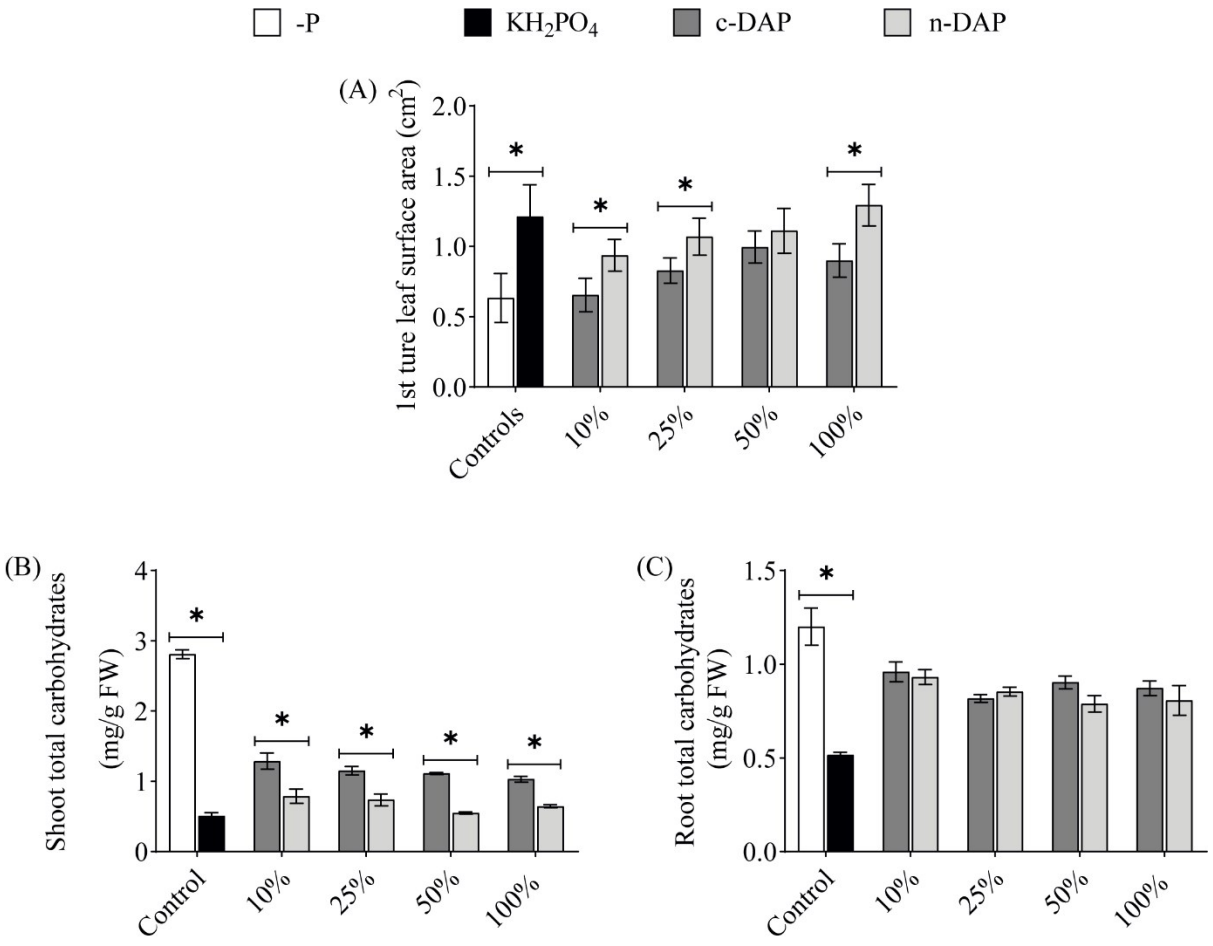

Fig. S4 Changes in (A) the surface area of the first true leaf (14 DAT), (B) shoot total carbohydrate content and (C) root total carbohydrate content of c-DAP and n-DAP supplemented *S. lycopersicum* seedlings on 15 DAT with respect to the controls are represented in graphs. The seedlings were grown by supplementing P-fertilizers by mixing with the solid growth media. Error bars indicate standard deviation (n≥15) and bar colours indicate the type of P used for the treatment. Asterisks indicate significance in increasing order in two-way ANOVA test (Sidak's multiple comparisons test) using GraphPad PRISM 8 where $P < 0.05$.

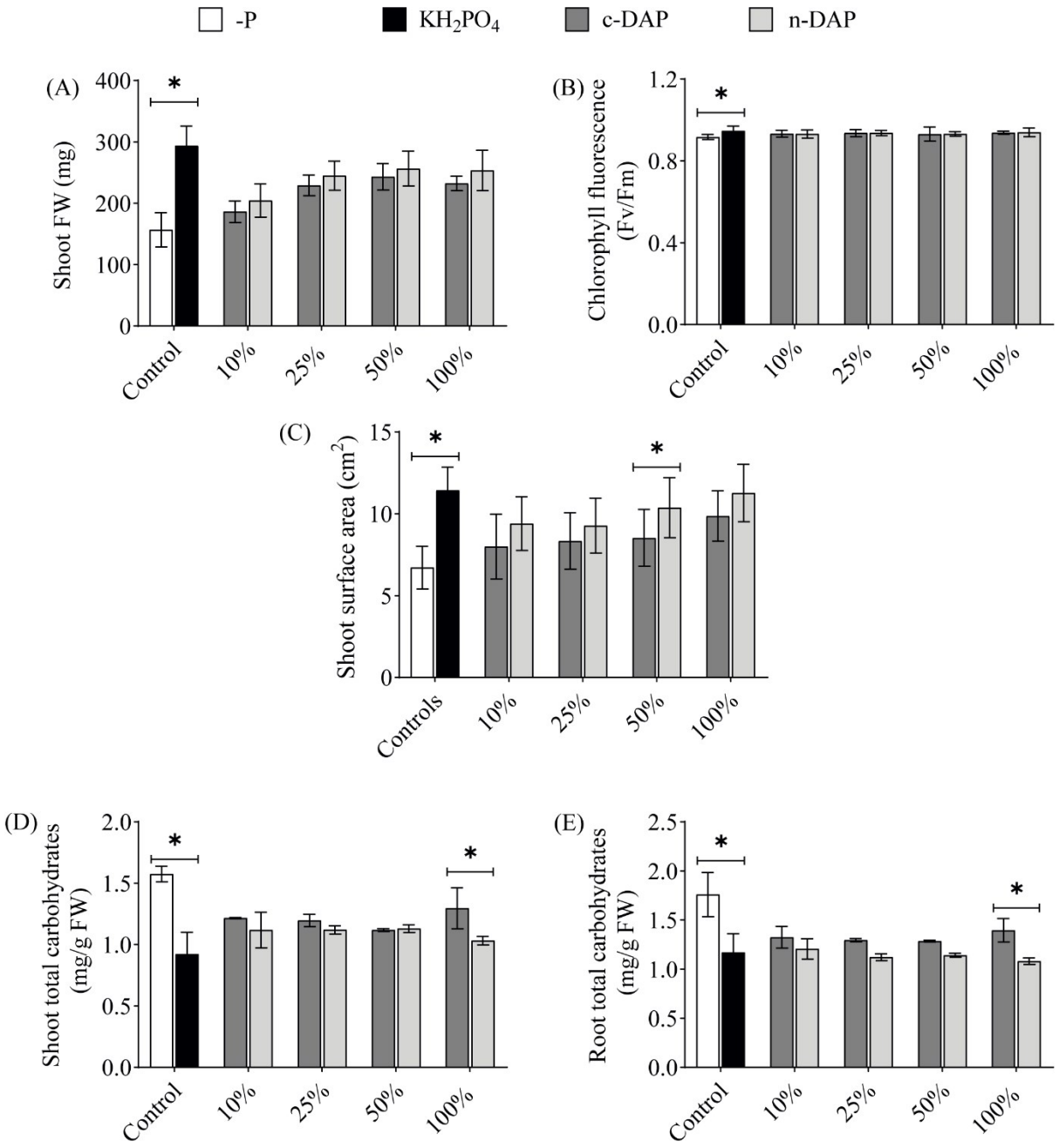

Fig. S5 Changes in (A) the shoot fresh weight, (B) chlorophyll fluorescence, (C) shoot surface area, (D) shoot total carbohydrate content and (E) root total carbohydrate content of c-DAP and n-DAP supplemented *Triticum aestivum* seedlings on 14 DAT with respect to the controls are represented in graphs. The seedlings were grown by supplementing P-fertilizers by mixing with the solid growth media. Error bars indicate standard deviation [($n \geq 10$ for A), ($n \geq 30$ for B, C), ($n \geq 15$ for D, E) and bar colours indicate the type of P used for the treatment. Asterisks

indicate significance in increasing order in two-way ANOVA test (Sidak's multiple comparisons test) using GraphPad PRISM 8 where $P < 0.05$.

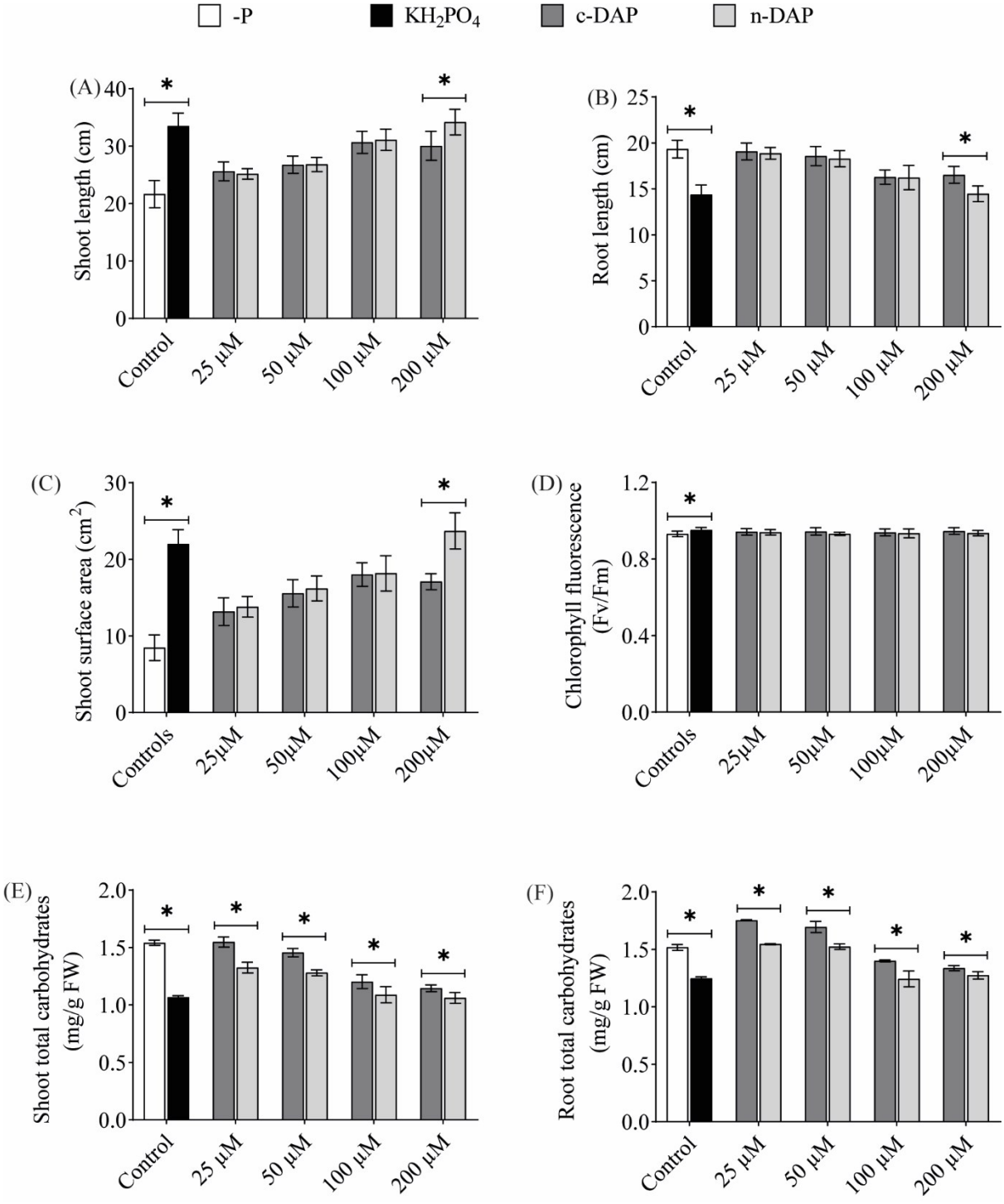

Fig. S6 Changes in (A) shoot length, (B) root length, (C) shoot surface area, (D) chlorophyll fluorescence, (E) shoot total carbohydrate content and (F) root total carbohydrate content of c-DAP and n-DAP supplemented *Triticum aestivum* seedlings on 14 DAT with respect to the controls are represented in graphs. The seedlings were grown hydroponically by supplementing P-fertilizers by mixing with P free manually prepared ½X MS media. Error bars indicate

standard deviation [($n \geq 30$ for A, B, C), ($n \geq 10$ for D), ($n \geq 15$ for E, E) and bar colours indicate the type of P used for the treatment. Asterisks indicate significance in increasing order in two-way ANOVA test (Sidak's multiple comparisons test) using GraphPad PRISM 8 where $P < 0.05$.