

# **Application of Biochar and Zeolite as a replacement for a peat-basef soilless potting substrate**

## **1. Pre-charging of Biochar & Zeolite with NPK:**

This experiment was conducted to determine the optimal concentrations of NPK (nitrogen, phosphorus and potassium) for charging Biochar and Zeolite. Biochar and zeolite were charged with 9 different combinations of NPK (Table 1) solution in 2:1 ratio (v/v) and agitated (200 rpm at room temperature) for 1 week. Charged Biochar and Zeolite were filtered and dried at 60°C overnight. The pH was measured and the charged Biochar and zeolite were analyzed for nutrients.

**Table 1:** NPK fertilizer charging solutions

Charging solutions	N (34-0-0) mg/L	P (0-42-0) mg/L	K (0-0-62) mg/L
NPK1	500	30	120
NPK 2	500	50	120
NPK 3	500	30	180
NPK 4	500	50	180
NPK 5	1000	30	120
NPK 6	1000	50	120
NPK 7	1000	30	180
NPK 8	1000	50	180
<b>NPK 9</b>	<b>1000</b>	<b>30</b>	<b>60</b>

**Note:** According to the data analysis, the NPK charging solution with a concentration of 1000-30-60 (1000mg/L of N, 30mg/L of P2O5 and 60mg/L of K2O) was found to be the most effective in charging Biochar and Zeolite. The ratio of NPK will be used to conduct the germination and growth experiments of basil and spinach.

## **1. Germination assays to select the optimal Biochar and zeolite combination in potting soilless substrate**

Germination assays in petri plates with basil seeds were conducted to find out the most suitable ratio combination of charged Biochar and zeolite. Potting soilless substrate made of Zeolite and Biochar at different % were mixed with peat in 3 different concentrations (Trt. 1, 2, 3, **Table 2**) and compared to peat alone (Control: Trt 5). Biochar or Zeolite alone or combined together (trt 4,6,7,8) were also used as substrate for germination assays.

**Table 2:** potting mix composition and germination test for Basil seeds.

Trt*	Media compositions	pH of the mixture	Germination rate (%)	Seedling length(mm)	Standard Error
1	22.5% BO + 10% ZO + 67.5% Peat	7.0	90%*	54	1.350
2	45% BO + 10% ZO+ 45% Peat	7.5	100%*	53	1.308
3	67.5%BO + 10% ZO + 22.5%Peat	8.5	96.6%*	47	1.258
4	90%BO + 10%ZO	9.5	96.6%	41	1.368
5	100% Peat **	4.3	76.7%	12	0.631
6	100% BO***	11.5	0%	0	0
7	100% ZO****	8	90%	47	0.973
8	10%ZO +90%Peat	5.2	90%	53	1.350

Trt\* – Treatments, \*\*Peat was not charged with NPK solution, therefore pH shown in table is of original peat. \*\*\* BO= Biochar; \*\*\*\*ZO=Zeolite, Stronger seedlings, dark color of first leaflets

Compared to the control treatment (Trt 5), % germination and total seedling length were higher in treatments 1 and 2 (22.5% BO:10% ZO and 45% BO:10% ZO). Compared to 100% peat (control), 100% Zeolite was also a good substrate for basil seed germination only (Table 2) but not for seedling growth. Interestingly, 100% Biochar (Trt 6) inhibited basil seed germination. Although basil seeds in treatments with higher biochar ratios (Trt, 2, 3 and 4) had high % seed germination, the seedlings eventually died.

**Note:** From the above results, the potting soilless mix containing **22.5 % Biochar +10% zeolite and 67.5% peat** was found the most effective.

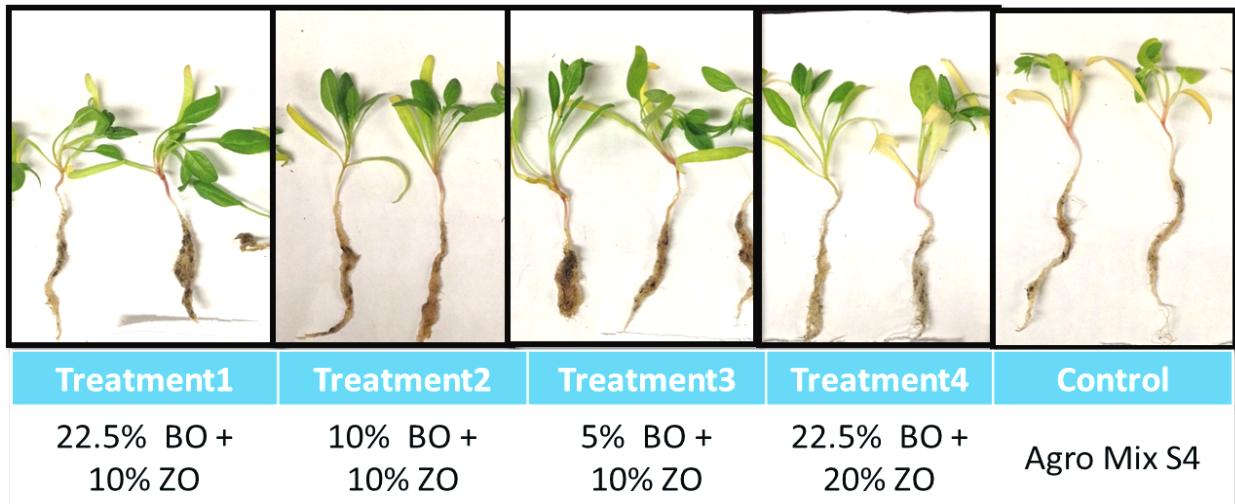
### **3. Tray experiment: Effect of pre-charged Biochar and zeolite on seed germination and seedling growth of Spinach & Basil**

This experiment was conducted using pre-charged biochar and Zeolite (NPK: 100-30-60) with peat (Agro mix S4) mixed in ratios as shown in **Table 3**. Peat 100% was used as the Control trt. We also tried lower % of biochar and higher % of zeolite combination (Table 3). The experiment consisted of three replicates per treatment (24 seeds/ replicate) for both plant species: spinach and basil. Germination rates (%), root and shoot weight (g), and shoot:root ratio (w/w) were recorded after 3 weeks of growth for spinach and after four weeks for basil, respectively.

**Table 3:** Five combinations of tested growth media.

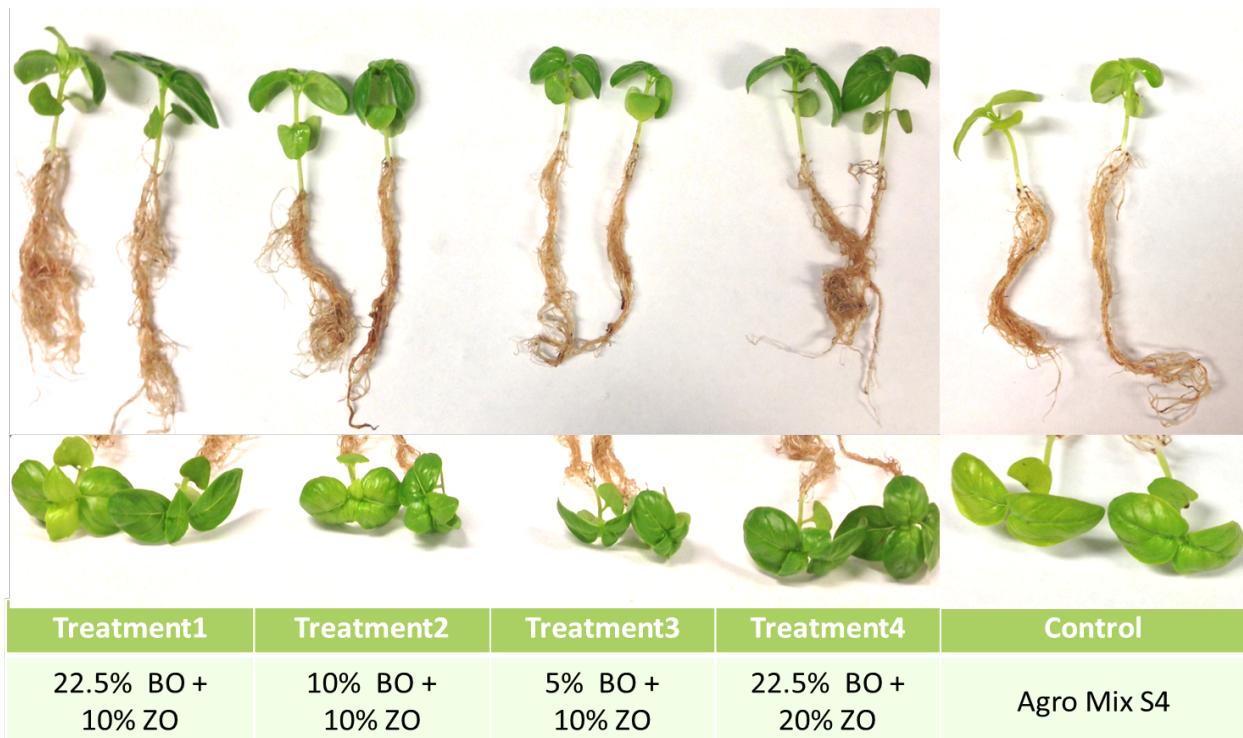
<b>Treatment</b>	<b>Charged Solution</b>	<b>Biochar</b>	<b>Zeolite</b>	<b>Agro mix (S4)</b>
		% (V/V)		
<b>1</b>	NPK solution (1000-30-60)	22.5	10	67.5
<b>2</b>	NPK solution (1000-30-60)	10	10	80
<b>3</b>	NPK solution (1000-30-60)	5	10	85
<b>4</b>	NPK solution (1000-30-60)	22.5	20	57.5
<b>AgroMix (Peat)</b>	-	0	0	100

There were no statistical significant differences in % germination of basil and spinach in different treatments (Data not shown). However, Spinach (Fig. 1A) and Basil (Fig. 1B) seedlings grown in treatments (1-4) were much healthier as reflected in their leaves and stems (e.g., leaves were dark green and stems were strong) when compared to the control (100% peat).



BO=biochar; ZO=zeolite

**Fig: 1A Spinach seedlings**



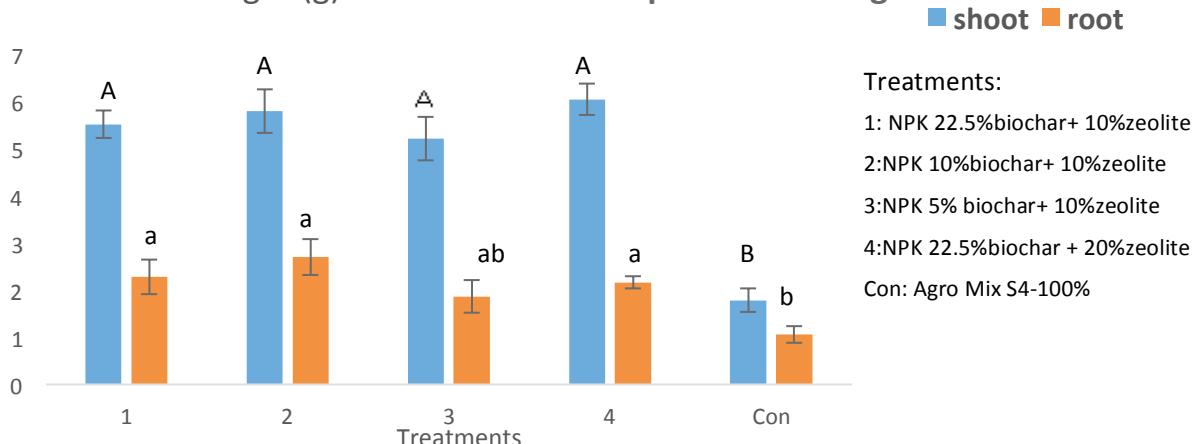
BO=biochar; ZO=zeolite

**Fig: 1B, Basil seedlings**

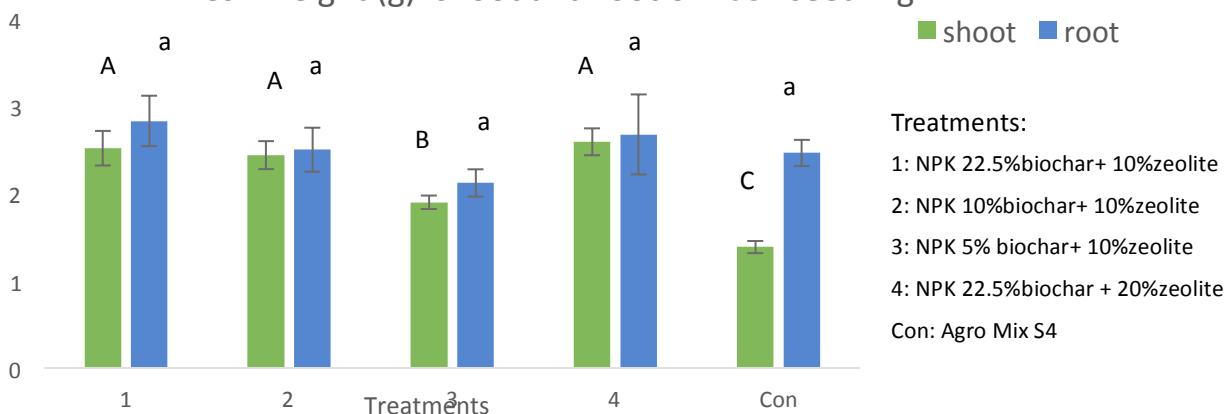
Fig.1A: Spinach seedlings in five germination media treatments, 1B: Basil seedlings in five germination media treatments (Each trial had 3 replicates per treatment (24 seeds/ replicate)).

**Fig: 2: Fresh weight of Shoot and root of Spinach (A) and Basil (B) seedlings**

**2A. Fresh weight (g): shoot and root of spinach seedlings**



**2B. Fresh weight (g): shoot and root of Basil seedling**



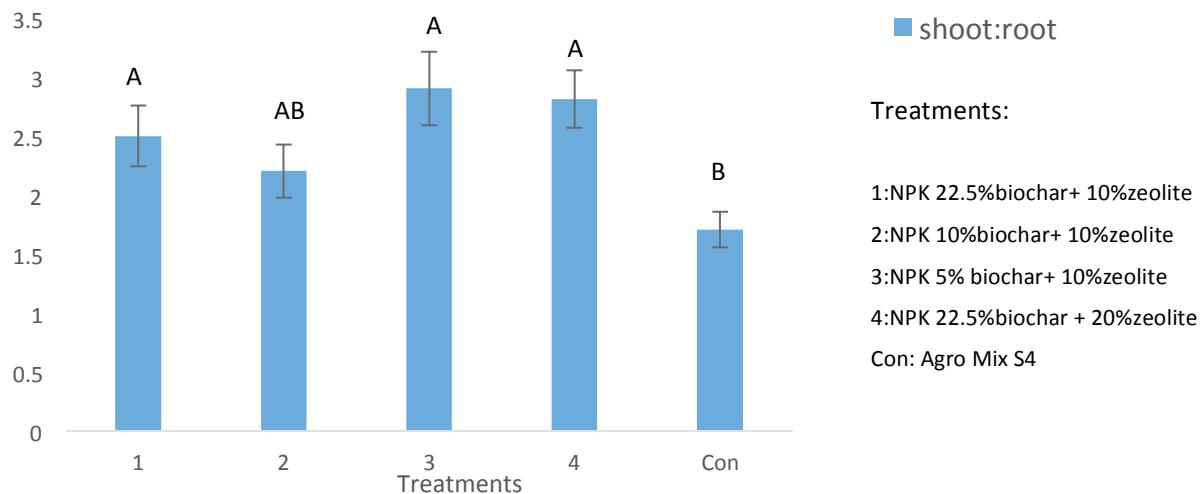
Bars represent the mean 3 replicates per treatment (24 seeds/ replicate).

**Fig. 2A.** The fresh weight of shoots of spinach seedlings grown in potting soilless substrate containing biochar and zeolite mixed with peat (Trt. 1-4) were significantly higher than those grown in peat alone (Control). The same trend was observed for root weight except for treatment 3.

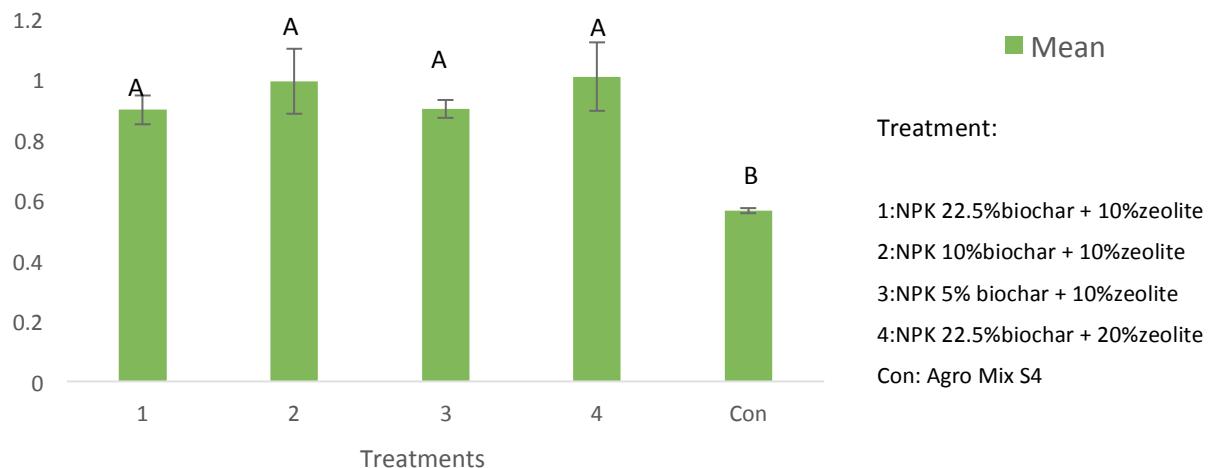
**Fig. 2B.** The fresh weight of shoots of basil seedlings grown in potting substrate containing biochar and zeolite mixed with peat (Trt. 1-4) were significantly higher than those grown in peat alone. Fresh root weight was not affected by biochar and zeolite treatment.

We have calculated the shoot:root ratio; this ratio is important for leafy vegetables and herbs. All seedlings grown in the pre-charged Biochar and Zeolite performed better than those grown in the control (3A & 3B). Moreover, the ratio observed in treatment 1, 3 and 4 are significantly higher than in control treatment. However, there was a treatment effect ( $P=0.0346$ ) in spinach.

**Fig: 3A. Spinach shoot:root (w/w) ratio**



**Fig. 3B. Basil Tray: Shoot:root (w/w) ratio**



### Summary of tray experiment

- Spinach and Basil:** Overall, seedlings germinated in pre-charged treatments have significantly higher shoot and root weights as well as shoot:root (w/w) compared to peat

alone, although there was no significance in % germination among treatments.

#### **4. Pot experiment: Effect of pre-charged Biochar & Zeolite on plant growth of Spinach & Basil in pots**

The two previous experiments concentrated on the effect of biochar and zeolite on germination and seedling growth for a short period of time. This experiment was designed to test the effect of pre-charged biochar and Zeolite combination on plant growth for a longer time period (2 -3 months) allowing the slow release of NPK fertilizers from the pre-charged Biochar and zeolite.

Spinach and basil seeds were sown in Agro mix® S4 in 200 cell trays for germination and after 3 weeks of growth, seedlings were transplanted into pots (6 inches). The 4 biochar and zeolite combination of potting mix substrates including peat alone as control (Table 3). For each species, there were five replicates for each potting mix substrate. Spinach plants were harvested after 30 days of transplanting and basil plants were harvested before flowering (40 days after transplanting). Fresh biomass of shoot and root were recorded (Figures 4 & 5).

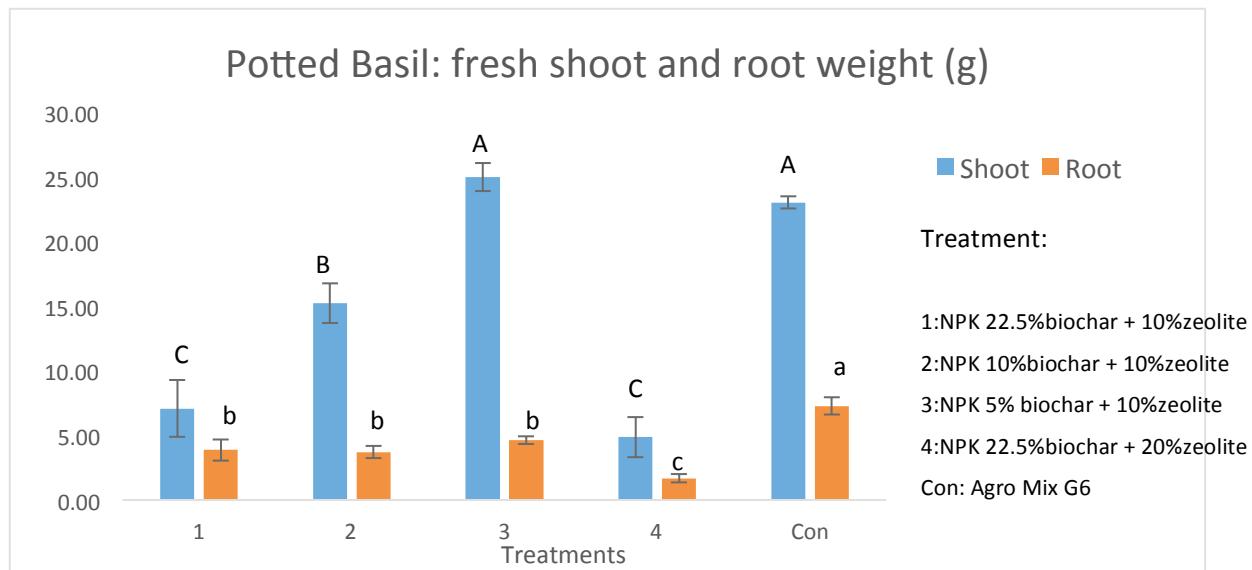
For basil plants, sugar content of leaves (Glucose kit), chlorophyll content, nutrient analysis and oxalic acid content (Oxalate kit) will be performed. A growth media analysis on pH, CEC, chlorophyll content and nutrient will also be performed.



BO=biochar; ZO=zeolite

Fig. 4: Basil plants grown in 5 growth substrates

**Fig.5:** Fresh shoot and root weight (g) of potted basil plants in 5 different substrates. Data obtained from five replicates.



**Results:** Figure 4 shows that Basil plants grown in treatments 2, 3 with pre-charged Biochar and zeolite were healthier and leaves were dark green as compared to control (peat alone) and also to treatment 4 (22.5% Biochar +20% zeolite). Plants grown in potting mix containing

biochar and zeolite higher than 10% had significantly lower shoot mass (Fig. 4, 5). Although plants in Trt 1 appeared healthy, plant growth was stunted

Although statistically not significant ( $P<0.001$ ), fresh shoot weight of basil plants grown in Treatment 3 (5% biochar +10% zeolite) was slightly higher than plants grown in 100% peat (control). Interestingly, the shoot:root ratio of basil plants in Treatment 3 was significantly higher than those grown in peat alone (Fig. 6). This is crucial for leafy vegetables such as basil as it is important to have bigger shoots.

Basil plants grown in peat alone (control) supported better root growth (fresh root weight) followed by those grown in potting mix containing 5% biochar and 10% zeolite.

Plants grown in treatment 3 (Fig. 5) have a healthier appearance (dark green leaves). To further confirm this effect chlorophyll content, nutrient analysis and some other test will be done soon.

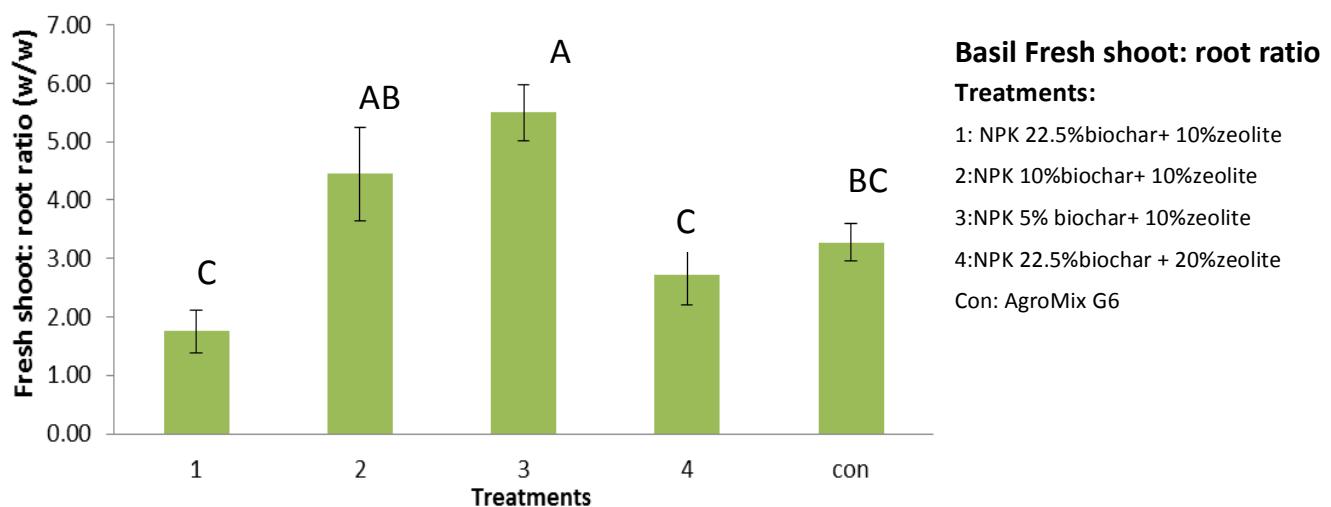
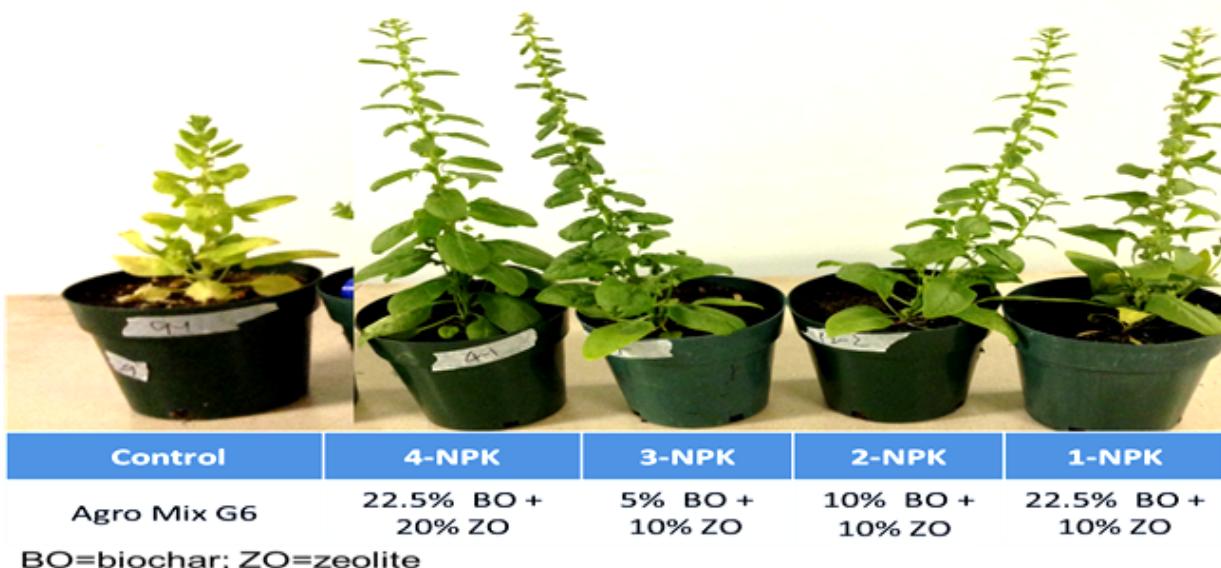


Fig: 6. Fresh shoot: root ratio (g) of potted basil plants in 5 growing Medias with five replicates Overall, 5% biochar + 10% zeolite supports the highest shoot: root ratio. Moreover, treatment effect ( $P=0.0006$ ) was very significant for fresh basil shoot: root ratio (w: w).

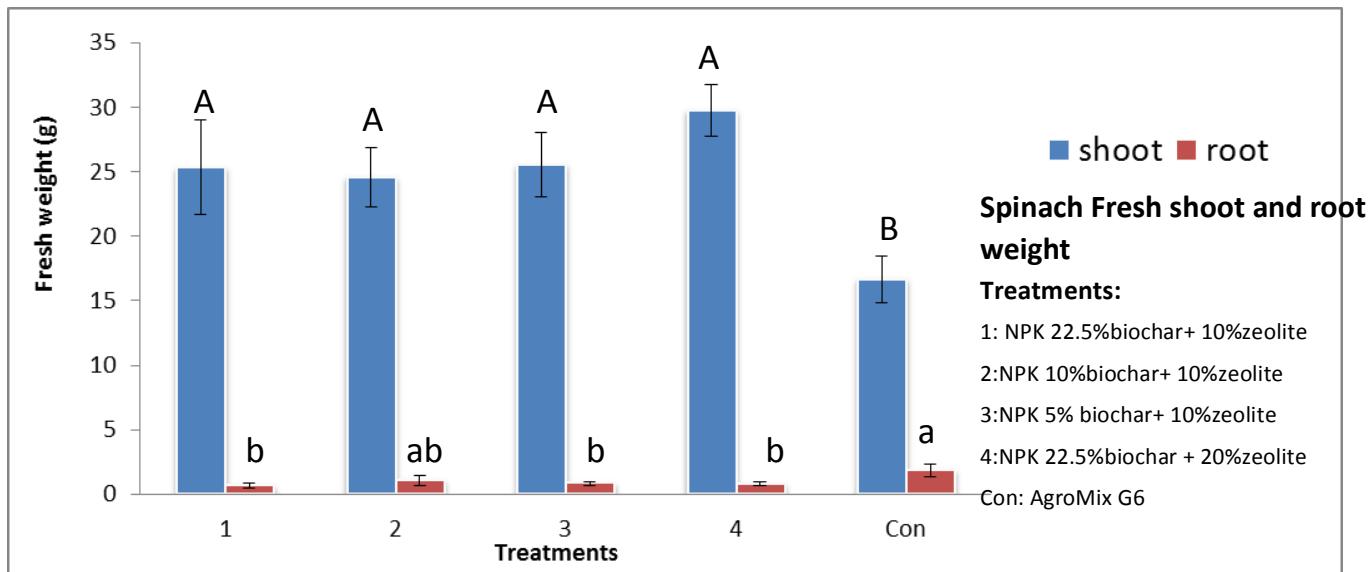
**SPINACH:** Plants grown in peat alone had yellow leaves, stunted growth and did not grow well

(Fig. 7) compared to those grown in peat moss amended with biochar and zeolite. These plants were healthier, their stems were longer and leaves were darker. Compared to the control, shoot weight of plants grown in peat amended with biochar and zeolite was significantly ( $P=0.0238$ ) higher (Fig. 8).

For Spinach, Fresh shoot weight in all treatments with charged biochar and zeolite are significantly higher than control indicating that pre-charged biochar and zeolite promote fresh shoot development. Similar to Basil, spinach plants in treatments containing biochar and zeolite were much taller and healthier than those grown in peat alone. NPK pre-charged potting substrate had a negative effect on root development (lower root weight). The Shoot: root ratio of spinach plants were significantly much higher than controls.



**Fig.7:** Spinach plants grown in five growth media, NPK; Treatment.



**Fig.8:** Fresh shoot and root weight (g) of potted spinach plants in 5 growing Medias. Data analyzed from five replicates.

### Summary.

Overall, the results clearly show that basil and spinach grown in pots containing peat amended with 5% biochar + 10% zeolite was the potting soilless substrate that produced healthier plants and generally high shoot mass and higher shoot:root ratios.

### **Note:**

*Spinach plants are sensitive to high temperature which in turn led to bolting in our experiments. We have observed huge fluctuation in the greenhouse temperature when conducting the experiments with Spinach. This has promoted the plant to go into the reproductive growth stage rather than vegetative growth. We request that this experiment be repeated under controlled conditions in growth chambers.*