

## Research Article

# Effect of salt (NaCl) stress on germination and early seedling growth of muskmelon (*Cucumis melo* L.)

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### Abstract

Salinity is one the most important constraints that limits crop production in arid and semi-arid region. Salinity influence and seed germination and it is the crucial for establishment of the plant that grow in saline soil. This research was carried out in order to test the effect of different salinity levels and growth of muskmelon, and the treatments were with different salt solutions at the, site Department of Horticulture, Garden Sindh Agriculture University Tandojam Pakistan, by the using Complete randomized design (CRD) with three replication including 4 levels of NaCl concentration (1,2,3, 4dsm<sup>-1</sup>) according to results high levels of seed germination %, number of leaves vine<sup>-1</sup>, seedling vigor index, vine length cm, fresh shoot biomass (g) and electrolyte leakage of leaf %. It seems that the including salinity up to 1 dSm<sup>-1</sup> improved vine length, seedling vigor index, and number of leaves vine<sup>-1</sup> and fresh shoot biomass (g) can be used for improving performance of Muskmelon and seedling growth under saline conditions.

**Keyword:** Against salinity tolerance; Germination; Muskmelon; Vegetative growth

### Introduction

Muskmelon (*Cucumis melo* L.) often called cantaloupes in the United States, are commonly grown here in Pakistan, although their cultivation is increasing in many areas of Sindh including all of the country. Its high water content help to stay hydrated during the hot season. Melon is divided into two groups namely Citrullus (Water melons) and Cucumis (muskmelon-cantaloupe group). The muskmelon is a member of the Cucurbitaceae family, which is muskmelon and popular crop with lots of medicinal values. It is considered as tonic

to heart and brain (laxative and diuretic) it is a good source of Vitamin A, B, and C and all its parts are used including the outer skin [1]. Muskmelon requires high temperature for successful growth and development and do not withstand temperature below 5 °C the optimum germination generally occurs at temperatures of 27-29 °C [2]. Hot dry air and sunshine during fruit ripening are needed for the development of high sugar content and good taste among the a biotic factors, salinity causes severe stress that limits crop productivity in the arid and semiarid areas, where the salt content of the

soils is higher and low precipitation and soil surface evaporation and transpiration, irrigation water salt concentration, excessive application of chemical fertilizer and lacking drainage system may be the factors that contribute to salinity stress. Worldwide, approximately 930 million hectares of land, 20 percent of the total agricultural land is under salinity stress [3]. Salinity adversely affected the physiological morphology and biochemical plant process such as the seed emergence plant growth and water nutrients uptake. The compared to other growth stage (flowering, fruiting), At this stage most vegetable crop exhibit sensitivity seven to low concentration of salt [4]. Saline causes such as adverse affected may be associate with low and the osmotic potential of soil solution ion affects inequality of nutrients to combined effects of all in worldwide in particular regions in many crop the seed germination and early seedling growth are the most sensitive stage to environments salt stress such as the seed germination and vegetative growth of muskmelon. In Pakistan almost 6.3 m h of land is affected by salinity which is estimated to be 14% of irrigation land this causes yield losses of 64% NaCl is the predominant salt causing salinization and it is expected that plant have involved in mechanism to regulate its accumulation in soil salinity may affected on seed germination as it reduce the moisture absorption of seeds and facilitating the entry of ions in higher amount that effects seed health. The levels of salinity at which seed germination is to reduce the different with species genotype environments and conditions of potential plants and ions [5]. The production of muskmelon is adversely too affected by high salinity levels in all over the world. The saline contributes in establishment of poor stand and eventually poor production of vegetable crops in saline soil it can become a limitation and factor for muskmelon establishments causing decrease in germination in rates [6].

In Sindh Pakistan the affects of salt stress NaCl on germination and early seedling growth of muskmelon is not well documented in this perspective it is mandatory to evaluate the effect of salt stress NaCl on seed germination and seedling growth of muskmelon.

#### **Materials and methods**

The pot experiment was carried at the experimental sit Horticulture Garden, Department of Horticulture Sindh Agriculture University Tandojam Pakistan, by using complete randomized design (CRD) with three replications and each replication contained three pots and in each pot four seeds were sown. The mixture of growing medium was filled in earthen pots leaving the approximately one inch space at the top in this research the seeds of two muskmelon varieties, Dhurga and Yellow pari were sown in pots the observations were recorded in each plant of both muskmelon.

#### **Statistical analysis**

The data were subjected to analysis using Statistics 8.1 computer software [7]. The least significance different LSD test was applied to compare treatments superiority in case results are significant at  $P \leq 0.05$  probability levels.

#### **Results**

In this study the effect of salt stress NaCl on germination and early seedling growth of muskmelon was to investigated the two muskmelon varieties ( $V_1$ = Durga and  $V_2$ = Yellow pari) were evaluated to against the different salinity levels ( $T_1$ = Control,  $T_2$  1 dsm<sup>-1</sup>,  $T_3$ = 2dsm<sup>-1</sup>,  $T_4$ = 3dsm<sup>-1</sup> and  $T_5$ = 4dsm<sup>-1</sup>) the data were recorded on the basis of certain number of plants some parameters of economics importance such as Germination (%), number of leaves vine<sup>-1</sup>, seedling vigor index, vine length (cm), fresh shoot biomass (g) and electrolyte leakage of leaf (%). The data on these traits of muskmelon are given in Tables 1-6 and results are described in view of the analysis of variance.

**Seed germination percentage (%)**

The germination percentage mainly reflects seed viability and for achieving a good crop stand, sowing of certified viable seed is of basic importance to achieve maximum seed germination in (Table 1). The analysis of variance (Appendix-I) depicted that seed germination of muskmelon was significantly influenced by varieties ( $F=5.11$ ,  $P=0.0363$ ,  $df=29$ ); while there was no significant variation in germination ( $P>0.05$ ) due to salinity levels ( $F=2.18$ ,  $P=0.1119$ ,  $df=29$ ) and varieties  $\times$  salinity levels interaction ( $F=1.30$ ,  $P=0.3091$ ,  $df=29$ ,  $CV=14.62\%$ ). The seed germination was significantly higher (74.81%) in muskmelon variety Durga as compared to variety Yellow Pari (66.29%). The salinity

effect showed that the seed germination was lowest (64.81%) in pots irrigated with 4  $dSm^{-1}$  saline water; while the seed germination increased to 66.66, 68.51 and 72.21 percent in pots irrigated with decreased salinity level up to 3  $dSm^{-1}$ , 2  $dSm^{-1}$  and 1  $dSm^{-1}$ , respectively. However, in control, where saline water was not used, the seed germination was highest (80.55%). The treatment interaction showed that variety Yellow Pari  $\times$  0 NaCl (control) resulted in highest seed germination (83.32%) and variety Yellow Pari  $\times$  4  $dSm^{-1}$  salinity resulted in lowest seed germination (55.55%). There was an inverse effect of salinity on the seed germination and with increasing salinity level, the seed germination was decreased considerably.

**Table 1. Effect of various salinity levels on Seed germination (%) of musk melon varieties**

Salinity levels	Varieties		Mean for salinity levels
	V1=Durga	V2=Yellow Pari	
T0= 0 (Control)	77.77	83.32	80.55
T1= 1 $dSm^{-1}$	77.77	66.66	72.21
T2= 2 $dSm^{-1}$	70.36	66.66	68.51
T3= 3 $dSm^{-1}$	74.07	59.25	66.66
T4= 4 $dSm^{-1}$	74.07	55.55	64.81
Mean for varieties	74.81A	66.29B	

S.E. 5.9555

LSD 0.05, 10.842

**Vine length (cm)**

Vine length may be a genetic characteristic of a particular variety, but the effect of soil type and soil quality cannot be ignored in (Table 2). The analysis of variance (Appendix-II) exhibited that vine length of muskmelon was significantly different in varieties ( $F=9.24$ ,  $P=0.0071$ ,  $df=29$ ), salinity levels ( $F=38.33$ ,  $P=0.0000$ ,  $df=29$ ) as well as by the varieties  $\times$  salinity levels interaction ( $F=6.34$ ,  $P=0.0023$ ,  $df=29$ ,  $CV=12.61\%$ ). The results showed that muskmelon variety Yellow Pari produced significantly longer vines (11.80 cm) than the variety Durga (10.26 cm). The salinity effect indicated that the vine length was least (6.08 cm) in pots irrigated with 4  $dSm^{-1}$  saline water; while the vine length

increased to 8.75 cm and 12.66 in pots irrigated with decreased salinity level up to 3  $dSm^{-1}$  and 2  $dSm^{-1}$ , respectively against the 12.99 cm vine length in control. However, the maximum vine length (14.66 cm) was recorded in pots irrigated with 1  $dSm^{-1}$  saline water. The treatment interaction showed that variety Yellow Pari  $\times$  1  $dSm^{-1}$  salinity resulted in maximum vine length (17.11 cm) and interaction of variety Durga  $\times$  4  $dSm^{-1}$  salinity resulted in lowest vine length (5.67 cm). The LSD test suggested similarity ( $P>0.05$ ) in vine length in pots irrigated with 0 NaCl (control) and 2  $dSm^{-1}$  saline water; while the differences were significant ( $P<0.05$ ) when these treatments were compared with rest of the salinity levels.

**Table 2. Effect of various salinity levels on vine length (cm) of musk melon varieties**

Salinity levels	Varieties		Mean for Salinity levels
	V1=Durga	V2=Yellow Pari	
<b>T0= 0 (Control)</b>	13.75	12.25	<b>12.99<sup>B</sup></b>
<b>T1= 1 dSm<sup>-1</sup></b>	12.22	17.11	<b>14.66<sup>A</sup></b>
<b>T2= 2 dSm<sup>-1</sup></b>	13.00	12.33	<b>12.66<sup>B</sup></b>
<b>T3= 3 dSm<sup>-1</sup></b>	6.67	10.83	<b>8.75<sup>C</sup></b>
<b>T4= 4 dSm<sup>-1</sup></b>	5.67	6.49	<b>6.08<sup>D</sup></b>
<b>Mean for varieties</b>	10.26 <sup>B</sup>	11.80 <sup>A</sup>	

S.E. 0.8033

LSD 0.05, 1.6876

**Seedling vigour index**

The seedling vigour index is calculated on the basis of length of vine  $\times$  percentage of seed germination. The analysis of variance (Appendix-III) demonstrated that seedling vigour index of muskmelon was significantly affected by salinity levels ( $F=12.41$ ,  $P=0.0001$ ,  $df=29$ ); while the variation in seedling vigour index was not significant ( $P>0.05$ ) between varieties ( $F=0.98$ ,  $P=0.3344$ ,  $df=29$ ) as well as for interaction of varieties  $\times$  salinity levels ( $F=0.97$ ,  $P=0.4501$ ,  $df=29$ ,  $CV=22.57\%$ ). It was observed from the results (Table 3). That seedling vigour index was relatively higher for muskmelon variety Yellow Pari (848.31) as compared to the variety Durga (781.69). The salinity effect showed that the seedling vigour index of muskmelon was highest (1073.50) in pots irrigated with saline water of 1 dSm<sup>-1</sup>; while the seedling

vigour index was relatively lower (1026.7) in control (0 NaCl). However, the seedling vigour index decreased to 885.4 and 637.0 in pots irrigated with saline water of 2 dSm<sup>-1</sup> and 3 dSm<sup>-1</sup>, respectively. However, the lowest seedling vigour index (452) was calculated in pots irrigated with saline water of 4 dSm<sup>-1</sup>. The treatment interaction showed that variety Yellow Pari  $\times$  1 dSm<sup>-1</sup> salinity resulted in maximum seedling vigour index (1134.4) and interaction of variety Durga  $\times$  4 dSm<sup>-1</sup> salinity resulted in lowest seedling vigour index (425.9). The LSD test described similarity in seedling vigour index ( $P>0.05$ ) in pots irrigated with 0 NaCl (control), 1 dSm<sup>-1</sup> and 2 dSm<sup>-1</sup> saline water; while the differences in seedling vigour index were also insignificant ( $P>0.05$ ) when 3 dSm<sup>-1</sup> and 4 dSm<sup>-1</sup> salinity levels were compared with each other.

**Table 3. Effect of various salinity levels on seedling vigour index of musk melon varieties**

Salinity levels	Varieties		Mean for Salinity levels
	V1=Durga	V2=Yellow Pari	
<b>T0= 0 (Control)</b>	1020.3	1033.2	<b>1026.7<sup>A</sup></b>
<b>T1= 1 dSm<sup>-1</sup></b>	1012.7	1134.4	<b>1073.5<sup>A</sup></b>
<b>T2= 2 dSm<sup>-1</sup></b>	949.6	821.2	<b>885.4<sup>A</sup></b>
<b>T3= 3 dSm<sup>-1</sup></b>	500.0	774.0	<b>637.0<sup>B</sup></b>
<b>T4= 4 dSm<sup>-1</sup></b>	425.9	478.8	<b>452.3<sup>B</sup></b>
<b>Mean for varieties</b>	781.69 <sup>A</sup>	848.31 <sup>A</sup>	

S.E. 106.20

LSD 0.05, 223.13

**Number of leaves vine<sup>-1</sup>**

The analysis of variance (Appendix-IV) illustrated that the number of leaves vine<sup>-1</sup> of muskmelon was significantly affected by

salinity levels ( $F=3.80$ ,  $P=0.0208$ ,  $df=29$ ); while the variation in number of leaves vine<sup>-1</sup> was not significant ( $P>0.05$ ) between varieties ( $F=3.56$ ,  $P=0.0357$ ,  $df=29$ ) as well

as for interaction of varieties  $\times$  salinity levels ( $F=1.95$ ,  $P=0.1464$ ,  $df=29$ ,  $CV=13.04\%$ ). The results presented in (table 4), revealed that the number of leaves per seedling was comparatively higher for muskmelon variety Durga (8.84) the leaves per seedling in variety Yellow Pari (4.42). The effect of salinity levels indicated that the number of leaves per seedling of muskmelon was highest (5.11) in pots irrigated with saline water of  $1 \text{ dSm}^{-1}$ ; while the number of leaves per seedling was equally higher (4.92) in control (0 NaCl) and in pots irrigated with saline water of  $2 \text{ dSm}^{-1}$ . However, the number of leaves per seedling decreased to 4.17 in pots irrigated with saline water of  $3 \text{ dSm}^{-1}$ ; while, the

lowest number of leaves per seedling (4.07) was recorded in pots irrigated with saline water of  $4 \text{ dSm}^{-1}$ . The treatment interaction showed that variety Durga  $\times$   $1 \text{ dSm}^{-1}$  salinity resulted in maximum number of leaves per seedling (5.72) and interaction of variety Yellow Pari  $\times$   $3 \text{ dSm}^{-1}$  salinity levels resulted in lowest number of leaves per seedling (3.66). The LSD test described similarity in number of leaves per seedling ( $P>0.05$ ) in pots irrigated with 0 NaCl (control),  $1 \text{ dSm}^{-1}$  and  $2 \text{ dSm}^{-1}$  saline water; and the differences in the number of leaves per seedling were also not significant ( $P>0.05$ ) when  $3 \text{ dSm}^{-1}$  and  $4 \text{ dSm}^{-1}$  salinity levels were compared.

**Table 4. Effect of various salinity levels on Number of leaves per seedling of musk melon varieties.**

Salinity levels	Varieties		Mean for Salinity levels
	V1=Durga	V2=Yellow Pari	
T0= 0 (Control)	5.00	4.83	4.92 <sup>A</sup>
T1= $1 \text{ dSm}^{-1}$	5.72	4.50	5.11 <sup>A</sup>
T2= $2 \text{ dSm}^{-1}$	5.00	4.83	4.92 <sup>A</sup>
T3= $3 \text{ dSm}^{-1}$	4.66	3.66	4.17 <sup>B</sup>
T4= $4 \text{ dSm}^{-1}$	3.83	4.30	4.07 <sup>B</sup>
Mean for varieties	4.84 <sup>A</sup>	4.42 <sup>A</sup>	

S.E. 0.3490

LSD 0.05, 0.7333

#### Fresh shoot biomass (g)

The fresh shoot biomass includes the entire weight of leaves, stem as well as the roots of the seedling. The analysis of variance (Appendix-V) depicted that the fresh shoot biomass of muskmelon was significantly affected by salinity levels ( $F=31.61$ ,  $P=0.0000$ ,  $df=29$ ); while the variation in fresh shoot biomass was not significant ( $P>0.05$ ) between varieties ( $F=0.17$ ,  $P=0.6824$ ,  $df=29$ ) as well as for interaction of varieties  $\times$  salinity levels ( $F=1.42$ ,  $P=0.2669$ ,  $df=29$ ,  $CV=17.69\%$ ). The results given in (Table 5), revealed that the fresh shoot biomass was relatively higher for muskmelon variety Yellow Pari (1.61 g) as compared to fresh shoot biomass of variety Durga (1.56 g). The salinity effect showed that the fresh seedling biomass of muskmelon was highest (2.34 g) in pots

irrigated with saline water of  $1 \text{ dSm}^{-1}$ ; followed by the fresh shoot biomass of 1.85 g and 1.80 g recorded in control (0 NaCl) and in pots irrigated with saline water of  $2 \text{ dSm}^{-1}$ , respectively. However, the fresh shoot biomass decreased to 1.31 g in pots irrigated with saline water of  $3 \text{ dSm}^{-1}$ ; while, the lowest fresh shoot biomass (0.84 g) was recorded in pots irrigated with saline water of  $4 \text{ dSm}^{-1}$ . The treatment interaction indicated that variety Durga  $\times$   $1 \text{ dSm}^{-1}$  salinity resulted in highest fresh shoot biomass (2.38 g) and interaction of variety Durga  $\times$   $4 \text{ dSm}^{-1}$  salinity levels resulted in lowest fresh shoot biomass (0.39 g). The LSD test suggested that the fresh shoot biomass was not significant ( $P>0.05$ ) in pots irrigated with 0 NaCl (control) and  $2 \text{ dSm}^{-1}$  saline water; and the differences in the fresh

shoot biomass were significant ( $P < 0.05$ ) compared with rest of the treatments.

**Table 5. Effect of various salinity levels on fresh seedling biomass (g) of musk melon varieties**

Salinity levels	Varieties		Mean for Salinity levels
	V1=Durga	V2=Yellow Pari	
0 (Control)	1.96	1.73	1.85 <sup>B</sup>
1 dSm <sup>-1</sup>	2.38	2.30	2.34 <sup>A</sup>
2 dSm <sup>-1</sup>	1.76	1.85	1.80 <sup>B</sup>
3 dSm <sup>-1</sup>	1.34	1.28	1.31 <sup>C</sup>
4 dSm <sup>-1</sup>	0.39	0.88	0.84 <sup>D</sup>
Mean for varieties	1.56 <sup>A</sup>	1.61 <sup>A</sup>	

S.E. 0.1622

LSD 0.05, 0.3408

#### Electrolyte leakage of leaf (%)

Electrolyte leakage is one of the main characteristics of stress response in intact plant cells and it is a measure of plant stress tolerance. The analysis of variance (Appendix-VI) revealed that the electrolyte leakage of leaf in muskmelon vines was significantly influenced by salinity levels ( $F=161.64$ ,  $P=0.0000$ ,  $df=29$ ) and varieties ( $F=32.78$ ,  $P=0.0000$ ,  $df=29$ ); while the variation in electrolyte leakage of leaf was not significant ( $P > 0.05$ ) for interaction of varieties  $\times$  salinity levels ( $F=0.20$ ,  $P=0.9341$ ,  $df=29$ ,  $CV=4.05\%$ ). It is evident from the results (Table 6), that the electrolyte leakage of leaf was significantly higher for muskmelon variety Durga (63.20%) than the electrolyte leakage of leaf in variety Yellow Pari (58.07%). The effect

of salinity levels showed that the electrolyte leakage of muskmelon leaf was highest (74.67%) in pots irrigated with saline water of 4 dSm<sup>-1</sup>; followed by the electrolyte leakage of 69.17%, 63.50% and 52.83% recorded in pots irrigated with saline water of 3 dSm<sup>-1</sup>, 2 dSm<sup>-1</sup> and 2 dSm<sup>-1</sup>, respectively. However, in control pots (0 NaCl) the electrolyte leakage of leaf decreased to lowest (43.00%). The treatment interaction indicated that variety Durga  $\times$  4 dSm<sup>-1</sup> salinity resulted in highest electrolyte leakage of leaf (77.00%) and interaction of variety Yellow Pari  $\times$  0 NaCl (Control) resulted in lowest electrolyte leakage of leaf (41.00%). The LSD test revealed that the electrolyte leakage of leaf was linear and significant ( $P < 0.05$ ) among the salinity levels.

**Table 6. Effect of various salinity levels on electrolyte leakage of leaf (%) of musk melon varieties**

Salinity levels	Varieties		Mean for Salinity levels
	V1=Durga	V2=Yellow Pari	
0 (Control)	45.00	41.00	43.00 <sup>E</sup>
1 dSm <sup>-1</sup>	56.00	49.66	52.83 <sup>D</sup>
2 dSm <sup>-1</sup>	66.00	61.00	63.50 <sup>C</sup>
3 dSm <sup>-1</sup>	72.00	66.33	69.17 <sup>B</sup>
4 dSm <sup>-1</sup>	77.00	72.33	74.67 <sup>A</sup>
Mean for varieties	63.20 <sup>A</sup>	58.07 <sup>B</sup>	

S.E. 1.4177

LSD 0.05, 2.9785

## Discussion

Salinity is the main constraint that limits crop productivity and particularly in arid and semi-arid areas. Seed germination is the most sensitive to salinity effect compared to other growth stages (flowering, fruiting). At this stage most vegetable crops exhibit sensitivity even to low concentration of salts impairs seed germination and reduces nodule formation and the germination percentage mainly reflects seed viability and for achieving a good crop stand, sowing of certified viable seed is of basic importance to achieve maximum seed germination [8]. The seed germination of muskmelon was significantly influenced by varieties; while there was not significant variation in germination due to salinity levels [9]. The seed germination was significantly higher (74.81%) in muskmelon variety Durga as compared to variety Yellow Pari (66.29%). The salinity effect showed that the seed germination was lowest (64.81%) in pots irrigated with 4 dSm<sup>-1</sup> saline water; while the seed germination increased to 66.66, 68.51 and 72.21 percent % in pots irrigated with decreased salinity level up to 3 dSm<sup>-1</sup>, 2 dSm<sup>-1</sup> and 1 dSm<sup>-1</sup>, respectively. However, in control, where saline water was not used, the seed germination was highest (80.55%). There was an inverse effect of salinity on the seed germination and with increasing salinity level, the seed germination was decreased considerably [10, 11]. Indicated that germination was hampered by the increasing salinity in vegetable crops including cucurbits. That initial stage of salinity near to acceptable limits may be slightly beneficial but the salinity always decreases the seed germination [12]. Suggested that for muskmelon the saline soils are not suitable for achieving desirable production. There are evidences of genetic influence on vine length and the crop varieties can produce more foliage but can be lower in production; while a variety genetically may be with less foliage but can be more promising in production. The vine length of muskmelon was significantly

different in varieties as well as under various salinity levels. Yellow Pari variety produced significantly longer vines (11.80 cm) than the variety Durga (10.26 cm). The salinity effect indicated that the vine length was least (6.08 cm) in pots irrigated with 4 dSm<sup>-1</sup> saline water; while the vine length increased to 8.75 cm and 12.66 in pots irrigated with decreased salinity level up to 3 dSm<sup>-1</sup> and 2 dSm<sup>-1</sup>, respectively against the 12.99 cm vine length in control. However [13], reported that the maximum vine length (14.66 cm) was recorded in pots irrigated with 1 dSm<sup>-1</sup> saline water. There was a substantial decrease in vine length with increasing the salinity level; while up to 1 dSm<sup>-1</sup> salinity level, the vine length was positively improved. These results are partially in agreement with those of who found negative effect of increasing salinity on the plant growth [14]. The similarly were of the experience that plant growth and development is adversely affected by increasing salinity beyond the tolerable limits.

## Conclusion

It is concluded that induced salinity up to 1 dSm<sup>-1</sup> improved vine length, seedling vigour index, and number of leaves per seedling and fresh seedling biomass but depressed germination. The salinity at 2, 3 and 4 dSm<sup>-1</sup> caused a simultaneous adverse effect on all the studied parameters except electrolyte leakage of leaf that increased parallel to the increasing salinity levels. Among muskmelon varieties, Yellow Pari surpassed variety Durga in germination percentage and electrolyte leakage of leaf; while variety Yellow Pari surpassed Durga in vine length, seedling vigour index and fresh shoot biomass. Statistical similarity ( $P > 0.05$ ) between varieties Dura and Yellow Pari was recorded for seedling vigour index, number of leaves vine<sup>-1</sup> and fresh shoot biomass.

## Author's contributions

Designed & idea the experiments: AQ Gola & MN Baloch, performed the experiments: MA Mahesar & JA Abro, implements the experiments: MS Mastoi & AQ Gola, Contributed reagents/ materials/ analysis

tools: MN Baloch & T Aziz, wrote the paper AQ Gola.

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