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MORPHOPHYSIOLOGICAL RESPONSE OF COTTON (*Gossypium* sp.) AGAINST NaCl INDUCED SALINITY STRESS

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Cotton (*Gossypium* sp.) is Bangladesh's second most important cash crop after jute. It is the main raw material of textile industry. The demand for cotton fiber in Bangladesh is increasing day by day and 8.5 million bales (480 pounds/bale) of cotton fiber were imported by expending $3.0 billion in the year 2021. The country's domestic cotton production in the cropping year 2021-22 was about 0.2 million bales with 6.35 bales ha^{-1} (Tabib, 2023).

Salinity is one of the major abiotic environmental stresses that affect the production of cotton. Salinity affects 20% of the world's land area and 33% irrigated agricultural area among which Asia is facing around 6% salinity of total land area which is estimated to increase at 10% rate annually (Arora *et al*., 2017; Sharif *et al*., 2019). Cotton is moderately salt-tolerant crop with salinity threshold level of 7.7 dS m^{-1} (Zhang *et al*., 2013). As a result, we could readily cultivate cotton in Bangladesh's saline region. In Bangladesh, there are 5000 hectares of saline land, of which it is possible to cultivate cotton on 2000 hectares (Uddin and Mortuza, 2015). Through ionic, oxidative and osmotic stresses the growth of cotton becomes inhibited in salinity stress (Zafar *et al*., 2022). Biochemical and physiological process of plant can also be hindered due to high salt concentration. Halting of metabolic process, root cell damage and generation of homeostatic environment are all results of the Na^+ ion accumulation (Farooq *et al*., 2020).

In order to expand the growth of cotton in Bangladesh, it is important to commence cultivation in every feasible location. The yield of cotton becomes significantly affected by poor germination and aberrant plant development under extreme salinized conditions though cotton is considered to be moderately salt tolerant crop. Growing salt-tolerant cotton cultivars on salt-affected soils is one way that cotton production succeeds (Chachar *et al*., 2008). Limited research has been conducted in Bangladesh about the impact of salinity on cotton growth. This study aimed to assess how salinity affected the growth and yield of cotton genotypes under saline soil conditions.

Pot experiment was conducted at Professor Purnendu Gain Field Laboratory of Agrotechnology Discipline of Khulna University, Bangladesh from September, 2019 to March, 2020 whose geographical location is 22.79904°N and 89.530677°E which belong to AEZ-13 (Ganges Tidal Floodplain). Two cotton genotypes (CB-9 previously reported as relatively salt-tolerant and CB-15 identified as susceptible one) were selected through the initial screening of germination parameters *in vitro* according to Mondal *et al*. (2020). These two genotypes were further evaluated to evaluate their comparative performance in pot conditions for growth and yield under different salt concentrations.

The pots were filled with 15 kg of well-drained clay loam soil (EC1:2 = 0.78 dS m^{-1}) collected from non-saline zone of Khulna division. To achieve the desired salinity levels, the required amount of salt (NaCl) was added and mixed properly to the soil except control one during soil preparation. After that, soil from each treatment was collected and examined by using an electrical conductivity (EC) meter to confirm the salinity level. Seeds of cotton were collected from Cotton Research, Training and Seed Multiplication Farm in Gazipur. In order to prevent soil-borne infections before planting, cotton seed was treated with Vitavex-200. Recommended doses of fertilizer (250:150:150 kg ha^{-1} of N, P_2O_5, and K_2O, respectively), appropriate weeding and irrigation was done using saline solutions of desired EC levels for treatments and good quality water for the control.

Scale measurements were made of the shoot and root lengths (cm) at 45 and 90 DAS. Dry weights (g) of the shoots and roots were measured following

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a 72-hour oven drying process at 70°C. 'ImageJ' software was used to compute the leaf area (cm^2). The following formula was used to calculate the relative water content (%) of leaves based on the ratio of fresh tissue weight to turgid tissue weight (Pieczynski *et al*., 2013).

$$\text{Leaf relative water content (\%)} = \frac{\text{Fresh weight(g)} - \text{Dry weight(g)}}{\text{Turgid weight (g)} - \text{Dry weight(g)}} \times 100$$

Chlorophyll 'a' and chlorophyll 'b' content was estimated by Arnon's equation (Arnon, 1949) where absorbance was read at 645 nm and 663 nm, respectively as:

$$\text{Chlorophyll 'a' content (mg/g)} = \frac{12.7(A663) - 2.69(A645)}{1000 \times w\ (\text{Fresh weight of the leaf sample})} \times v$$

$$\text{Chlorophyll 'b' content (mg/g)} = \frac{22.9(A645) - 4.68(A663)}{1000 \times w\ (\text{Fresh weight of the leaf sample})} \times v$$

Cotton boll from each plant was harvested and yield (kg/ha) was calculated.

$$\text{Ginning out turn was calculated as: GOT (\%)} = \frac{\text{Lint weight (g)}}{\text{Seed cotton weight (g)}} \times 100$$

According to Asana and Williams (1965), relative performance of root, shoot length, weight, chlorophyll content, yield was computed using the formula below:

$$\textit{Relative performance} = \frac{\textit{Variable measured under stress condition}}{\textit{Variable measured under normal condition}}$$

The experiment was laid out in a two-factor Completely Randomized Design (CRD) with three replications, where factor A consisted of four salinity treatments (Control, 4 dS m^{-1}, 8 dS m^{-1}, and 16 dS m^{-1}), and factor B consisted of two cotton genotypes (CB-9 and CB-15). 'STATISTIX 10' software was used to analyze the recorded data, and Tukey's HSD test was used to separate means at 5% level of significance.

Shoot and root length (cm)

Shoot and root length of both cotton genotypes (CB-9 and CB-15) declined progressively with increasing salinity stress compared to the control. At 45 DAS, CB-9 exhibited a higher shoot length (51.83 cm) under control, which gradually decreased to 32.33 cm at 16 dSm^{-1}, whereas CB-15 recorded 48.17 cm under control and the lowest value (25.83 cm) under 16 dSm^{-1}. A similar declining trend was observed at 90 DAS, where CB-9 maintained relatively greater shoot length (86.67 cm in control and 63.33 cm at 16 dSm^{-1}) compared to CB-15 (83.50 cm in control and 53.83 cm at 16 dSm^{-1}). In case of root length, genotypes showed significant variation only at 16 dS m^{-1} at both 45 and 90 DAS (Table 1). At 16 dSm^{-1}, CB- 9 showed 35 and 40% reduction while CB- 15 showed 52 and 53% reduction of root length respectively at 45 and 90 DAS.

Dry weight of shoot and root (g)

Shoot dry weight showed no significant difference between genotypes upto 8 dS m^{-1}. At 45 DAS, CB-9 produced 8.64 g in control, which reduced to 3.33 g at 16 dSm^{-1}. CB-15 showed a similar trend, with 8.27 g in control declining drastically to 1.77 g at 16 dSm^{-1}. At 90 DAS, CB-9 exhibited the highest shoot dry mass under control (33.24 g), but it declined to 9.42 g at the highest salinity. In CB-15, shoot dry weight decreased from 32.11 g in control to only 5.68 g at 16 dSm^{-1}.

Besides, dry weight of root showed significant variation between genotypes at 4 and 16 dS m^{-1}. At 45 DAS, CB-9 had 1.29 g under control, which decreased to 0.47 g at 16 dSm^{-1}. CB-15 declined more sharply from 1.23 g under control to 0.19 g at 16 dSm^{-1}. At 90 DAS, CB-9 maintained higher root biomass (4.40 g in control and 1.85 g at 16 dSm^{-1}), while CB-15 decreased from 3.59 g in control to only 1.06 g under severe salinity. (Table 1). This indicates that salinity significantly reduces biomass accumulation, and CB-9 retains comparatively higher shoot biomass than CB-15 under saline stress.

Leaf area (cm^2)

Relative to control treatment, leaf area at 16 dS m^{-1} was reduced less in CB-9 (66%, 54%) than in CB-15 (75%, 68%) at 45 DAS and 90 DAS respectively (Fig. 1A, 1B).

Leaf relative water content (%)

At 45 DAS, relative water content at 16 dS m^{-1}

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Table 1. Effect of salinity on shoot and root length (cm) and dry weight (g) of cotton at 45 DAS and 90 DAS

Salinity level (dSm^{-1})	Genotype	Shoot length (cm)		Root length (cm)		Shoot dry weight (g)		Root dry weight (g)	
		45 DAS	90 DAS	45 DAS	90 DAS	45 DAS	90 DAS	45 DAS	90 DAS
Control	CB-9	51.83^{a}	86.67^{a}	18.50^{a}	27.00^{a}	8.64^{a}	33.24^{a}	1.29^{a}	4.40^{a}
	CB-15	48.17^{ab}	83.50^{a}	17.01^{a}	24.17^{ab}	8.27^{a}	32.11^{a}	1.23^{a}	3.59^{ab}
4	CB-9	47.16^{b}	81.00^{ab}	15.02^{b}	20.67^{bc}	7.52^{ab}	25.50^{ab}	0.97^{bc}	3.07^{b}
	CB-15	41.17^{c}	75.33^{bc}	14.12^{bc}	19.67^{c}	6.76^{b}	19.19^{bc}	0.82^{cd}	2.62^{cd}
8	CB-9	40.00^{c}	70.00^{cd}	13.15^{cd}	18.33^{cd}	5.19^{c}	14.91^{cd}	0.63^{de}	2.35^{cd}
	CB-15	32.75^{d}	63.67^{d}	11.95^{d}	17.67^{cd}	4.07^{cd}	12.67^{cd}	0.54^{e}	2.18^{d}
16	CB-9	32.33^{d}	63.33^{d}	11.93^{d}	16.17^{d}	3.33^{d}	9.42^{d}	0.47^{e}	1.85^{d}
	CB-15	25.83^{e}	53.83^{e}	8.12^{e}	11.33^{e}	1.77^{e}	5.68^{e}	0.19^{f}	1.06^{e}

Within a column, means followed by different alphabet are significantly different after Tukey's HSD All pairwise test at $p \leq 0.05$

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was higher in CB-9 (0.76) than in CB-15 (0.67). At 90 DAS, it was also higher in CB-9 (0.61) than in CB-15 (0.56) at 16 dS m^{-1} (Fig. 1C, 1D)

Chlorophyll 'a' content (mg/g FW)

Relative to control treatment, chlorophyll 'a' content at 16 dS m^{-1} was reduced less (32%) in CB-9 and more (50%) in CB-15 at 45 DAS. At 90 DAS, the highest reduction was recorded in CB- 15 (62%) than CB- 9 (48%) at 16 dS m^{-1} (Fig. 1E, 1F).

Chlorophyll 'b' Content (mg/g FW)

The minimum reduction of chlorophyll 'b' (40%) was found in CB-9 at 16 dS m^{-1} in comparison to the control treatment and maximum (70%) in CB-15 at 45 DAS. At 90 DAS, the highest reduction was recorded in CB- 15 (76%) than CB- 9 (59%) at 16 dS m^{-1} (Fig. 1G, 1H).

Number of bolls and single boll weight (g)

No significant difference observed upto 8 dS m^{-1} between genotypes but at 16 dS m^{-1} the lowest number of bolls (4.67) was found in CB-15 which was statistically different from other treatments. Similar pattern result was found in case of single boll weight. The lowest single boll weight (2.65 g) was found in CB-15 at 16 dS m^{-1} which was statistically different from other treatments (Table 2).

Yield (kg ha^{-1})

Up to 8 dS m^{-1}, no significant yield difference recorded between genotypes but at 16 dS m^{-1}, CB-15 had the lowest yield (322.3 kg ha^{-1}), which was apparently different from the other results (Table 2).

Ginning outturn (%)

The control of CB-9, had the highest ginning outturn (43.08%), which was statistically identical to the control of CB-15 (40.89%) and CB-9 (38.24 %) at 4 dS m^{-1}. CB-15 had the lowest GOT (21.06%) at 16 dS m^{-1}, which was statistically distinct from other treatments (Table 2).

The present study demonstrated that increasing salinity levels significantly suppressed shoot and root growth, as well as biomass accumulation, in both cotton genotypes. Such reductions are consistent with earlier reports that salinity impairs plant growth through osmotic stress, ion toxicity, and nutrient imbalance (Ibrahim *et al.*, 2019). The decline in shoot length under salinity may be attributed to reduced cell expansion and division caused by osmotic stress, while the suppression of root length reflects the adverse effects of excess Na^{+} and Cl^{-} on root meristem activity as well as salinity lowers roots' capacity to absorb water, which may be due to reduced Ca^{2+} along with cation exchange activity in the soil (Chachar *et al.*, 2008). The maintenance of higher shoot and root length in CB-9 (Table 1) indicates better osmotic adjustment and more efficient water uptake under saline conditions. Root length, in particular, is a critical trait for salinity adaptation, as deeper and longer roots improve access to moisture and enhance ion exclusion (Chen *et a*l., 2022). Ibrahim *et al*. (2019) also supported our results and found that salinity decreased the height of plant, root length, and both the dry and fresh weight of stems, roots, and leaves of cotton considerably.

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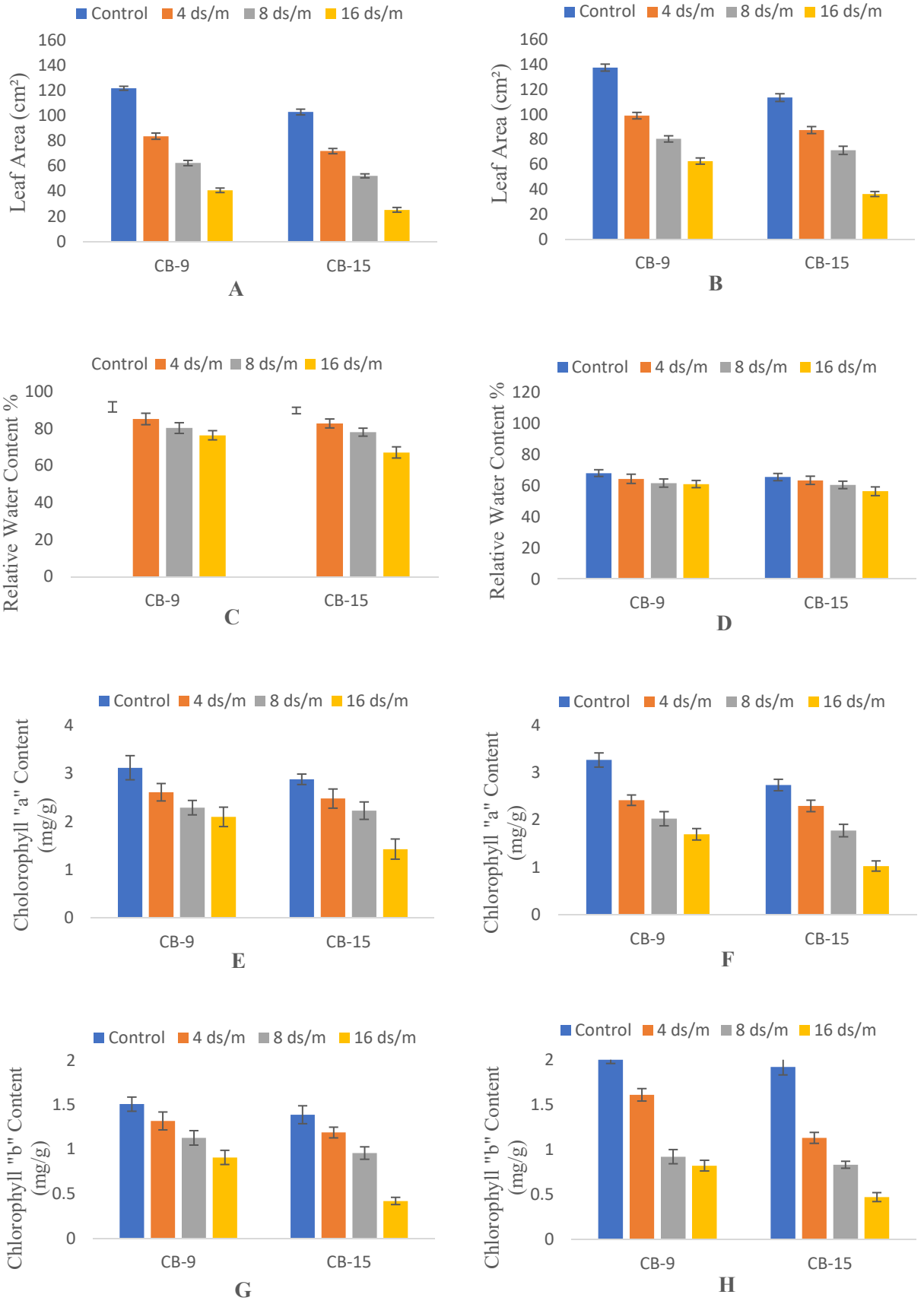


Fig. 1. Leaf area (cm²), relative water content (%), chlorophyll 'a' and 'b' content (mg/g FW) of two cotton genotypes at different salinity level (Control, 4, 8, 16 dS m^{-1}) at 45 DAS (A, C, E, G) and 90 DAS (B, D, F, H)

In our experiment, leaf area and leaf relative water content showed a decreasing tendency along with increasing salinity in both genotypes but decreasing tendency was more prominent in CB-15 than in CB-9 (Fig. 1). Sikder *et al.* (2020) found that the water potential lowers and prevents the roots from absorbing water during salt stress, which lowers the relative water content of the leaves. Reduced photosynthetic production per plant due to a reduction in total leaf area is one of salinity's primary consequences. Enhanced assimilate distribution to the roots at the expense of leaf development was a major contributing factor to the loss in leaf area (Sharif *et al*., 2019). Thus, more decrease in growth metrics seen in the stressed plants of CB-15, may be attributed to the osmotic impact brought on by the salt stress, which disrupted the water balance of the plants, resulting in stomatal closing, a decrease in photosynthesis, and ultimately a growth inhibition (Farooq *et al*., 2020)

According to our study, under soil salinity stress, chlorophyll 'a' and chlorophyll 'b' was reduced more in CB-15 indicating its susceptibility to salinity. Under low salinity stress, stomatal closure was the primary cause of the decrease in chlorophyll content; however, as soil salinity increased, non-stomatal limitations such as an increase in the activities of sucrose-metabolizing enzymes and the degradation of chlorophyll became the primary causes of chlorophyll reduction and photosynthetic limitation (Peng *et al*., 2016).

The present study also indicated that there were significant differences in yield contributing characters between the two cotton genotypes as salt increased and CB- 9 performed better compared to CB-15 (Table-2). The quantity of bolls per plant varied dramatically between cotton genotypes as salt increased. Since a decrease in plant growth resulted in a reduction in the number and development of sympodia (fruit-producing shoots), it suggests that a rise in salt content had a stressful impact on cotton blooming and boll production. The mass of seed cotton on each boll varied greatly amongst cotton genotypes, same as other metrics. A higher quantity of salt directly reduced the weight of the boll. It is thought that the photosynthesis rate, which is clearly correlated with the intake of nutrients and moisture from the soil, is directly proportional to the boll weight. Since rising saline levels have a substantial impact on yield-contributing elements, reduced cotton yield is the outcome (Phogat *et al*., 2001).

CB-9 consistently outperformed CB-15 across all morphophysiological traits, suggesting a relatively greater tolerance to salt stress. The decline in all morphophysiological traits under salinity is in line with the reduced photosynthetic activity and impaired assimilate partitioning reported in salt-stressed plants. However, the relatively higher biomass retention in CB-9 compared to CB-15 implies more efficient photosynthetic performance and possibly better

Table 2. Effect of salinity level on number of bolls, single boll weight (g), yield (kg/ha) and ginning out turn (%) of cotton

Salinity level (dS m^{-1})	Genotype	Number of bolls $plant^{-1}$	Single boll weight (g)	Yield/ plant (g)	Ginning outturn (%)
Control	CB-9	25.33^{a}	5.36^{a}	133^{a}	43.08^{a}
	CB-15	24.33^{a}	5.28^{a}	121.9^{ab}	40.89^{a}
4	CB-9	21.00^{ab}	5.19^{a}	121.6^{ab}	38.24^{ab}
	CB-15	19.67^{bc}	4.90^{ab}	100.8^{bc}	33.74^{bc}
8	CB-9	16.00^{cd}	4.82^{ab}	$85.4.2^{cd}$	31.32^{c}
	CB-15	14.00^{de}	4.40^{b}	72.2^{d}	29.21^{c}
16	CB-9	11.00^{e}	3.47^{c}	44.7^{e}	28.52^{c}
	CB-15	4.67^{f}	2.65^{d}	17.9^{f}	21.06^{d}

Within a column means followed by different alphabets differ significantly after Tukey's HSD All Pairwise test at $p \leq 0.05$

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antioxidant defense, which are common adaptive responses in tolerant genotypes (Hasanuzzaman and Fujita, 2022). In contrary, the sharp reduction in all traits of CB-15 further highlights its lower capacity for sustaining metabolism and ion homeostasis under salt stress.

Taken together, the superior performance of CB-9 may be linked to its ability to maintain osmotic balance, restrict toxic ion accumulation, and preserve assimilate production under salinity stress. These findings highlight CB-9 as a relatively salt-tolerant genotype, selecting in optimizing cotton yield in saline affected area and offering potential for use in breeding programs aimed at improving salinity tolerance in cotton.

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Authors' contribution

Conceptualization of research work and designing of experiments (JR,, MYA), Execution of field experiment and data collection (SA, NJ); Analysis of data and interpretation (JR, PKD), Preparation of manuscript (JR, NJ).

Conflict of interest

The authors declare that there is no conflict of interest.

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