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Research Article

RESPONSE OF RELATIVE LEAF WATER CONTENT, CHLOROPHYLL STABILITY INDEX, PROLINE, AND YIELD OF COTTON TO THE APPLICATION OF BIOCHAR, MULCH AND PPFM SPRAY UNDER DIFFERING MOISTURE REGIMES

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Abstract- A Field experiment was conducted at Agricultural College and Research Institute, Madurai during Winter Irrigated Cotton Season of 2016-17 with the test variety SVPR − 4. To study the effect of moisture regimes on physiological parameters of Relative Leaf Water Content (RLWC), Chlorophyll Stability Index (CSI), Proline and Seed cotton yield. Experiment was laid out in split plot design with three replications. Moisture regimes were assigned to the main plots *viz*., Irrigation at IW/CPE ratio to 0.4 (I_1) and 0.8 (I_2). The subplot comprises with moisture management practices with Biochar application *viz*., B_1-Cotton stalk biochar @ 5 t ha $^{-1}$, B_2-Cotton stalk biochar @ 5 t ha^{-1} + Crop residue mulch @ 5 t ha^{-1}, B_3-Cotton stalk biochar @ 5 t ha^{-1} + Crop residue mulch @ 5 t ha^{-1}+ PPFM @ 500 ml ha^{-1} on 75 and 90 DAS, B_4- *Prosopis* biochar @ 5 t ha^{-1}, B_5- *Prosopis* biochar @ 5 t ha^{-1} + Crop residue mulch @ 5 t ha^{-1}, B_6 - *Prosopis* biochar @ 5 t ha^{-1}+ Crop residue mulch @ 5 t ha^{-1} + PPFM @ 500 ml ha^{-1} on 75 and 90 DAS and B_7 − Control. The results of this study showed that RLWC, CSI of Cotton were recorded higher and lower values of Proline were recorded under irrigation at IW/CPE to 0.8 (I_2) and *Prosopis* biochar @ 5 t ha^{-1}+ Crop residue mulch @ 5 t ha^{-1} + PPFM @ 500 ml ha^{-1} on 75 and 90 DAS (B_6) . The same treatments registered for higher values of crop yield.

Keywords- Moisture Stress, Chlorophyll stability index, Biochar, Cotton.

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Introduction

Cotton is a perennial shrub that has been cultivated by man for several thousand years. Cotton fiber is amazingly versatile, whether alone or blended, it out sells all other fibres combined. It is a soft, absorbent, and breathable natural fibre, making it the perfect for clothing. Cotton, truly a miracle fibre, it has been spun, woven, and dyed since ancient times, and it is still the most widely used and preferred fibre for cloth today. India is topmost cotton producing country with 11.88 mha^{-1} area under production accounting 30 per cent of world coverage and 22 per cent (351 lakh bales of lint) of the world cotton production and productivity of 568.29 kg ha^{-1} [1]. It is estimated that more than 5.8 million farmers cultivate cotton in India and about 40-50 million people are employed directly or indirectly by the cotton industry. Considering this importance of cotton crop different attempts have been made to boost up its production.

Cotton is best grown in soils with an excellent water holding capacity. The moisture stress is the primary cause for the yield reduction in Cotton. Water scarcity and rapid environmental changes force us to find a way to conserve the available soil water and properly manage it for efficient crop production. In the way Biochar application helps in several ways, by improving soil moisture retention and conserving water in the soil. It holds to secure the crops against moisture stress.

Biochar is the porous carbonaceous solid produced by thermo chemical conversion of organic materials in oxygen depleted atmosphere [2]. Biochar application into soil may increase the overall net soil surface area consequently may improve the soil water retention [3] and soil aeration. With that the adoption of Surface cover with organic mulches is an effective *in-situ* moisture conservation practice. Hatfield *et al*., [4]. reported that there was 34- 50 % reduction in soil water evaporation because of crop residue mulching.

And also, the Pink Pigmented Facultative Methylobacteria (PPFM) Spray which improve the crop stand to moisture stress. PPFM release the osmoprotectants (sugars and alcohols) on the surface of host plants. This matrix may help to protect the plants from desiccation and high temperatures [5] mad. Keeping this in view, an attempt was made to study the effect of biochar, mulch and PPFM spray on agronomic response of cotton under moisture stress condition.

The physiological parameters such as Relative leaf water content (RLWC) [6], Leaf proline accumulation [7] and Chlorophyll stability index (CSI) [8] are some sensitive physiological indicators used to study the response of plants under moisture stress condition.

The RLWC is a measure of the amount of water present in the leaf tissue. Leaf RWC is one of the best growth/biochemical index to reveal the stress intensity [9]. Sampathkumar [10] reported that cotton plants irrigated at full irrigation levels maintained higher RLWC than moisture-stressed treatments in the experiment. Kar *et al.* [11] noted the response of different cotton cultivars to moisture stress and found that moisture stress apparently increased the proline levels. The stability of chlorophyll under moisture stress was expressed as CSI. Moisture stress damaged the cell membrane and affected the stability of chlorophyll [12].

Materials and Methods

A field experiment was conducted at Central Farm, Department of Farm Management, Agricultural College and Research Institute, Madurai during *Winter Irrigated Cotton Season* of 2016-17. The experimental site falls under the Southernagro-climatic sub-zone of Tamil Nadu and located at 9°54' N latitude and 78°80' E longitude at an altitude of 147 m above mean sea level. The mean annual rainfall is 786.6 mm in 40 rainy days. The mean maximum and minimum temperature of the location are 33.4˚C and 23.6˚C respectively. The relative humidity ranges from 60 to 80 per cent. The soil of the experimental plot was Sandy clay loam in texture, Neutral in pH, medium in organic carbon, low in available nitrogen, medium in available phosphorus and high in available potassium. The experiment was laid out in Split plot design, replicated thrice with test variety SVPR 4.

The main plots consist of two different moisture regimes *viz.,* I_1- IW/CPE ratio of 0.4 and I_2- IW/CPE ratio of 0.8 and Moisture management practices with Biochar application in subplots *viz*., B_1-Cotton stalk biochar (CSB) alone @ 5 t ha $^{-1}$, B_2-Cotton stalk biochar @ 5 t ha^{-1} + Crop Residue Mulch (CRM) @ 5 t ha^{-1}, B_3-Cotton stalk biochar @ 5 t ha^{-1} + Crop residue mulch @ 5 t ha^{-1}+ PPFM @ 500 ml ha^{-1} on 75 and 90 DAS, B_4- *Prosopis* biochar alone @ 5 t ha^{-1}, B_5- *Prosopis* biochar @ 5 t ha^{-1} + Crop residue mulch @ 5 t ha^{-1}, B_6 - *Prosopis* biochar @ 5 t ha^{-1}+ Crop residue mulch @ 5 t ha^{-1} + PPFM @ 500 ml ha^{-1} on 75 and 90 DAS and B_7 – Control.

The observations were recorded in the five fully expanded leaves of the treatment plots which is of the canopy fully exposed to sunlight were selected at random and tagged for assessing the physiological parameters. Observations were made before irrigation cycles during at 70, 90 and 105 DAS of cotton crop.

i). RLWC was estimated from the method suggested by Barrs and Weatherly [13] and result was expressed in percentage.

$$RLWC\ (\%) = \frac{\text{Leaf Fresh Weight} - \text{Leaf Dry Weight}}{\text{Leaf Turgid Weight} - \text{Leaf Dry Weight}} \times 100$$

ii). CSI was assessed according to the method suggested by Murty and Majumder[14] and result was expressed in percentage.

iii). The leaf proline accumulation was estimated by [15]. The quantity of proline in the test sample was calculated with reference to standard curve and expressed in terms of µmol g⁻¹ FW.

Iv). The seed cotton yield was obtained from net plot area was shade dried, weighed at each picking and yields of all picking were added and calculated as kg per plot and then expressed in kilogram per hectare. The data obtained were subjected to statistical analysis and were tested at five per cent level of significance to interpret the treatment differences as suggested by [16].

Results and Discussion

The moisture stress is a major factor in reducing plants growth, development, and productivity. The physiological responses of plants under moisture stress noted with RLWC, CSI, Proline, and yield. It is well known that water stress limits the crop production by reducing yield levels with induced physiological changes inside the crop plants. In this study also, water stress had significant effect on crop yield.

Effect of different moisture regimes and moisture conservation practices with biochar on Physiological parameters

Leaf Relative water content and

Moisture regimes had profound influence on RLWC and registered higher values during the flowering (70 DAS) and comparatively less during the later stages of 90 and 105 DAS [Table-1]. The earlier 70 DAS of cotton RLWC was higher under Irrigation at IW/CPE ratio to 0.8 (I_2) with 81.8 % and lower was recorded in irrigation at IW/CPE ratio to 0.4 (I_1) with 73.4 %. The same trend was followed for the later stages of RLWC with irrigation at IW/CPE ratio to 0.8 (I_2) on 90 and 105 DAS with 72.1 and 80.2 respectively. The lower values were recorded under Irrigation at IW/CPE ratio to 0.4 (I_1) at 90 and 105 DAS with 68.0 and 76.0 % respectively. The observed significant decrease in RLWC under moisture stressed condition was due to reduced absorption of water from the soil and inability to control water loss through the stomata. Our results are in assenting the findings of Kumar *et al*. [17], Ananthi *et al*. [18]. In the case of CSI, the increased levels were recorded with IW/CPE ratio of 0.8 (I_2) during 90 and 105 DAS with 58.6 and 59.0 % respectively [Table-1]. A higher CSI helps the plants to withstand moisture stress through better availability of chlorophyll. The sufficient moisture level in the plant root zone might be the reason for higher CSI [19].

Table-1 *Effect of different moisture regimes and moisture conservation practices with biochar on Leaf Relative water content and chlorophyll stability index*

Treatments			RWC (%) 70 DAS	RWC (%) 90 DAS	RWC (%) 105 DAS	CSI (%) 90 DAS	CSI (%) 105 DAS
Moisture Regimes							
I_1	-	IW/ CPE ratio of 0.4	73.4	72.1	68.0	50.3	52.0
I_2	-	IW/ CPE ratio of 0.8	81.8	80.6	76.0	58.6	59.0
	S.Ed		1.20	1.27	1.24	0.89	1.04
	CD (p=0.05)		5.18	5.45	5.34	3.84	4.47
Moisture Conservation Practices							
B1	-	CSB	74.6	72.5	68.8	53.5	52.6
B2	-	CSB+CRM	78.5	77.7	73.0	53.5	55.1
B3	-	CSB+CRM+PPFM on 75 and 90 DAS	78.5	79.7	74.2	55.1	56.7
B4	-	PB	77.5	74.6	70.3	54.5	53.3
B5	-	PB+CRM	80.5	79.7	75.4	56.1	59.2
B6	-	PB+CRM+PPFM on 75 and 90 DAS	80.5	81.0	77.3	58.1	61.2
B7	-	Control	72.9	69.1	65.4	50.5	50.4
	S.Ed		1.78	1.02	1.14	1.07	1.04
	CD (p=0.05)		3.68	2.11	2.36	2.21	2.14
Interaction : I ×B							
	S.Ed		2.62	1.84	1.95	1.66	1.71
	CD (p=0.05)		NS	5.75	5.78	4.50	4.94

*CSB- Cotton Stalk Biochar, CRM- Crop Residue Mulch,
PPFM-Pink Pigmented Facultative Methylobacteria, PB – *Prosopis* biochar

Among the moisture conservation practices with biochar application *Prosopis* biochar with crop residue mulch at 5t/ha + Foliar application of PPFM (500 ml/ha) at 75 and 90 DAS (B_6) recorded for the higher RLWC of 81.0 and 77.3 % at 90 and 105 DAS respectively. The same treatment recorded for higher CSI at 90 and 105 DAS with 58.1 and 61.2 % respectively. Which is comparable with *Prosopis* biochar with crop residue mulch at 5t/ha (B5) at 90 and 105 DAS for both RLWC and CSI. The surface area of the biochar was very high and was highly porous in nature with variable charge organic material that had the potential to increase soil water holding capacity in soil [20] with that addition of mulch can improvise the soil moisture by arresting evaporation with modified microclimate levels may reduce the leaf and soil temperature [21]. In addition to this PPFM spray with that treatment release the osmoprotectants (sugars and alcohols) on the surface of the plants. This matrix may help to protect the plants from desiccation and high temperatures [5].

Leaf Proline accumulation

Water stress induces a significant decrease in metabolic factors such as decrease in chlorophyll content and enhanced accumulation of proline [22]. Accumulation of proline is a widespread plant response to environmental stress, including low water potential. Proline accumulation is believed to play adaptive roles in plant stress tolerance [23]. Moisture regimes had a positive increase in the Leaf Accumulated Proline levels. When the moisture stress was getting high the proline levels also recorded high. The moisture regime irrigated at IW/CPE ratio to 0.4 recorded for high levels of Proline in 70, 90 and 105 DAS with 10.05, 9.82 and 8.23 µmol g^{-1} FW respectively [Table-2]. Minimum amount of proline was noticed with the irrigation at IW/CPE to 0.8. In the case of moisture conservation practices with biochar application *Prosopis* biochar with crop residue mulch at 5 t/ha + Foliar application of PPFM (500 ml/ha) at 75 and 90 DAS (B_6) recorded for the lower levels of proline at 90 and 105 DAS. This was comparable with *Prosopis* biochar with crop residue mulch at 5t/ha (B_5). Proline content of leaves increased with decline in irrigation water inferred that the production of proline is probably a common response of crops under water-stressed condition. The accumulation of free proline in stressed plants has been found to be an adaptive mechanism for drought tolerance and a positive correlation between magnitude of free proline accumulation. In this study also moisture stressed treatments recorded the higher proline levels. This is in conformity with the findings of [19,24,25].

Table-2 *Effect of different moisture regimes and moisture conservation practices with biochar on Leaf proline accumulation*.

Treatments			PROLINE (µmol g−1 FW)		
			70 DAS	90 DAS	105 DAS
Moisture Regimes					
I_1	-	IW/ CPE ratio of 0.4	10.05	9.82	8.23
I_2	-	IW/ CPE ratio of 0.8	5.79	7.39	5.67
	S.Ed		0.18	0.39	0.24
	CD (p=0.05)		0.74	1.66	1.04
Moisture Conservation Practices					
B1	-	CSB	8.53	10.15	825
B2	-	CSB+CRM	7.55	7.77	6.25
B3	-	CSB+CRM+PPFM on 75 and 90 DAS	7.55	7.55	5.90
B4	-	PB	7.75	9.11	7.65
B5	-	PB+CRM	6.83	7.33	6.10
B6	-	PB+CRM+PPFM on 75 and 90 DAS	6.85	6.46	5.36
B7	-	Control	10.38	11.83	9.15
	S.Ed		0.82	0.40	0.24
	CD (p=0.05)		1.69	0.83	0.50
Interaction : I ×B					
	S.Ed		1.09	0.65	0.40
	CD (p=0.05)		NS	1.86	1.16

*CSB- Cotton Stalk Biochar, CRM- Crop Residue Mulch,
PPFM-Pink Pigmented Facultative Methylobacteria, PB – Prosopis biochar

Table-3 *Effect of different moisture regimes and moisture conservation practices with biochar on Seed cotton yield*

Treatments			Seed cotton yield (kg/ha)
Moisture Regimes			
I_1	-	IW/ CPE ratio of 0.4	1399
I_2	-	IW/ CPE ratio of 0.8	1677
	S.Ed		47
	CD (p=0.05)		204
Moisture Conservation Practices			
B1	-	CSB	1373
B2	-	CSB+CRM	1484
B3	-	CSB+CRM+PPFM on 75 and 90 DAS	1581
B4	-	PB	1472
B5	-	PB+CRM	1787
B6	-	PB+CRM+PPFM on 75 and 90 DAS	1963
B7	-	Control	1107
	S.Ed		64
	CD (p=0.05)		132
Interaction : I ×B			
	S.Ed		96
	CD (p=0.05)		251

*CSB- Cotton Stalk Biochar, CRM- Crop Residue Mulch,
PPFM-Pink Pigmented Facultative Methylobacteria, PB – Prosopis biochar

Effect of different moisture regimes and moisture conservation practices on seed cotton yield

Moisture stress made significant yield differences between the treatments. Irrigations at IW/CPE ratio to 0.8 produced significantly higher seed cotton yield of 1677 kg/ha over Irrigation at IW/CPE ratio to 0.4 reached 1399 kg/ha [Table-3]. The increase in seed cotton yield could be attributed to greater and consistent available soil moisture due to increased level of irrigation that resulted in better crop growth yield and physiological components. In case of moisture conservation practices with biochar application *Prosopis* biochar with crop residue mulch at 5 t/ha + Foliar application of PPFM (500 ml/ha) at 75 and 90 DAS (B_6) recorded for higher seed cotton yield 1963 kg/ha. In combination the Irrigation at IW/CPE ratio to 0.8 and *Prosopis* biochar with crop residue mulch at 5 t/ha + Foliar application of PPFM (500 ml/ha) at 75 and 90 DAS recorded higher seed cotton yield. This was evidenced from the values recorded for critical physiological characters, viz. RLWC, proline accumulation in leaf and CSI.

Conclusion

The higher RLWC and CSI indicates the better availability of water in the cell, which increase the photosynthetic rate, dry matter production and high productivity. Also the higher range of proline content in leaves were recorded under moisture stressed treatments, suggesting that the production of proline is probably a common response of crops under water-stressed condition. Under moisture stressed regime irrigation at IW/CPE to 0.4 (I_2) is well managed with *Prosopis* biochar with crop residue mulch at 5 t/ha (B_5) and in addition Foliar application of PPFM (500 ml/ha) at 75 and 90 DAS (B_6) for the better yield.

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Author Contributions

1. Kannan V, Ph.D. Scholar in the Dept. of Agronomy, AC & RI, Madurai. He has doing research work in stress management of cotton.

2. Srinivasn G, Scientist who gave valuable suggestions for the research work. He has specialized in weed management and Cotton.

3. Babu R, Scientist has specialized in water management.

4. Thiyageswari S, Scientist has specialized in Soil Biology, plant nutrition and soil mineralogy.

5.Sivakumar T, Scientist has specialized in Stress Physiology

Abbreviations

DAS: Days After Sowing
RLWC: Relative Leaf Water Content
CSI: Chlorophyll Stability Index
PPFM: Pink Pigmented Facultative Methylobacteria

Conflicts of Interest: None declared.

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