Effect of thiourea as root growing medium on growth, photosynthetic pigments, ions and yield attributes of cotton under drought stress

Misbah[1], Muhammad Shahbaz[1]

[1]Department of Botany, University of Agriculture, Faisalabad, Pakistan

Abstract

Water scarcity condition decreases the yield production of most important crops which leads alarming situation for food security worldwide. Drought stress reduces the growth and development up to 70% in different plants. Exogenously applied thiourea protected the plants by improving growth and yield in different plants. A pot experiment was conducted to estimate the role of thiourea as rooting medium treatment on different aspects of growth, yield, gas exchange, photosynthetic pigments, and availability of mineral nutrients under moisture stress condition in two varieties (FH-114 and FH-118) of cotton (*Gossypium hirsutum* L.) plant. After 49 days of sowing, the plants were applied with two drought stress treatments (normal watering and 60% field capacity) and three level of thiourea (water, 10 mM and 20 mM) applied after two weeks of drought stress (after 63 days of sowing) as root growing medium for this experiment. The completely randomizes design was used with each treatment replicated four times. In this research, water scarcity condition considerably decreased the growth parameters (such as shoot length, shoot fresh and dry weight), gas exchange parameters [such as net CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance (g_s) and water use efficiency (A/E)], photosynthetic pigments (such as chlorophyll *a*, total chlorophyll and carotenoid contents), mineral ions (like potassium, calcium and sodium of shoot) and yield attributes (such as number of bolls and seeds, weight of bolls and seeds and number of sympodial branches). Among different level of thiourea, 10 mM as root growing medium significantly improved the rate of CO_2 assimilation (A), conductance of stomata (g_s), water use efficiency (A/E) and yield attributes. While application of 20 mM thiourea increased the fresh and dry weight of shoot, stomatal conductance, chlorophyll *a* contents, total chlorophyll contents, mineral ions uptake of shoot and yield attributes. Among different levels of thiourea, 20 mM was better than other levels in enhancing the growth, photosynthetic pigments and yield attributes. Variety FH-142 performed better as compared to FH-942.

Keywords: Cotton, drought**,** thiourea, ions, yield

I. INTRODUCTION

Various types of environmental stresses reduce development of different crops by altering their life cycle (Zandalinas *et al*., 2018). Low moisture condition and high temperature are the serious threats for global food safety because it significantly decreases the growing ability of major foods (Alghabari *et al*., 2015). Water scarcity condition is one of the main reason which ultimately restrictive the different aspects of development and total yield production of different plants (Alam *et al*., 2021). Water scarcity condition decreases the soil fertility upto 90% which leads to the loss of development of staple food upto 70% (Mantri *et al*., 2012). Water stress condition disturbed about 33% of world agriculture land and decreases the growth of major crops (Nawaz *et al*., 2021). Shortage of water condition decreases the development of plants by disturbing the size of leaves and activities of different enzymes which take part in morpho-physiological processes (Hameed *et al*., 2021). Among different stresses, water scarcity condition affects the different aspect of plant such as in maize decreases leaf area, water use efficiency and also changed the process of opening and closing of stomata which ultimately declined the process of growth in different plants (Alghabari *et al*., 2015). Low water availability condition also disturbed the ionic balance which required for the different developmental attributes of plants (Yasmin *et al*., 2021). Water scarcity condition significantly decreases the production of yield by disturbing the different enzymes activity (Mumtaz *et al*., 2021). Water scarcity condition also decreases the yield of different crops by enhancing the rate of boll exfoliating and also due to the fruit discoloration (Zlatev and Lidon, 2012).

Low water availability condition decreases the development process of different plants by producing the oxygen free radicals (Heile *et al*., 2021). Under water scarcity condition, oxygen radicals are produced in excess quantities which affect the development process of plant by disturbing the enzyme activities which involved in the photosynthetic process (Basu *et al*., 2016). The shortage of water condition significantly decreases the developmental process of peanut (Furlan *et al*., 2012). Low water availability condition decreases the developmental and yield parameters of legumes by lowering the number and size of flowers and seeds (Fang *et al*., 2010). The growth of chickpea up to 33% significantly diminishes by the negative effects of low water availability condition (Kashiwagi *et al*., 2015). Low water availability condition significantly decreased the growth and yield production by lowering the photosynthetic pigments (Parveen et al., 2021).

Among different cash crop, *Gossypium hirsutum* L. (cotton) is the major cash and uncertain crop which is most important due to its high fiber value (Constable and Bange, 2015). Water scarcity condition badly affects the plant growth by causing lessening in the development and yield of cotton plant (Brown *et al*., 2003). Cotton grows in China, India, USA and Pakistan due to hot climate (Riaz *et al*., 2013). Low water availability condition significantly decreases the 34% of cotton yield as compared to previous year (Dawn news, 2016). In past (over the last 50 years), water stress condition decreases the yield of cotton and other important crops up to 67% (Comas *et al*., 2013). Low water availability condition decreases the growth of cotton plant by decreasing height of plant, leave and stem dry weight, number of nodes, growth of root and quality of fiber (Loka *et al*., 2011). In *Gossypium barbadense*, water scarcity condition significantly decreases the photosynthesis and transpiration process and also decreases the dry matter accumulation up to 50% (Hejnak *et al*., 2015).

For the removal of adversarial effects of different abiotic stresses, plant growth regulators plays important role. Among different plant growth regulators, thiourea is most important growth regulators under different environmental stresses due to the presence of nitrogen and Sulphur in it (Garg *et al*., 2006). Thioure is most effective in the removal of bad effects of low water avaiability condition in different crops by improving the photosynthetic process and thus increasing the yield production (Baqer *et al*., 2020). Application of thiourea as root growing medium in potato significantly improved the different pigments of photosynthesis (such as chl *a*, *b* and total chlorophyll) (Saleem *et al*., 2022). Thiourea increases the germination rate by breaking the seed dormancy (Esashi *et al*., 1977). Externally applied thiourea increases the fresh and dry weight in potato cultivars (Saleem *et al*., 2022). Application of thiourea showed helpful effect on improving the developmental and yield production in many plants by improving the gaseous exchange (Yadav *et al*., 2020). Under low water availability condition, using of thiourea as soaking of seed treatment, foliar spray and rooting medium improves the growth by improving defense mechanisms (Seleiman and Kheir, 2018). Water scarcity condition decreases the growth of plant while thiourea showed positive effects on the process of development by accumulative the photosynthetic process and defense mechanisms (Kaya *et al*., 2019). Externally applied thiourea enhanced the growth and yield production by improving the enzyme activities and osmolytes which removed the adverse effects of oxygen free radicals which are the main aspects for decreasing the developmental and yield production (Naz *et a*l., 2021). Under scarcity of water condition, externally applied thiourea stimulates the gene expression of ion channels, antioxidants and also for hormonal regulation which ultimately increases the different aspects of developmental and yield production of different plants (Patade *et al*., 2012). By the adjustment of microRNAs and hormones, thiourea increases the gene expression of defense mechanisms (Srivastava *et al*., 2017).

Under water scarcity condition, application of thiourea enhances the yield in cereals and pulses by enhancing the phloem loading and unloading mechanism (Singh and Singh, 2017). Low water availability stress decreases the growth and development in wheat plant. Externally applied thiourea improves the growth of plant by improving the photosynthetic process, respiration, ions uptake and loading and unloading of phloem mechanism (Abdelkader *et al*., 2012). Water scarcity condition decreases the gene expression for defense system and RbcL (Rubisco large subunit) while application of thiourea stimulates the gene expression for Rubisco large subunit which ultimately improves the photosynthetic activity in various crops (Vineeth *et al*., 2016). Low water availability condition decreases the water consumption up to 48.8% in onion. Exogenously applied thiourea enhances the growth and bulb yield by enhancing the water use efficiency (Wakchaure *et al*., 2018).

The main objectives of the studies were to explored the role of thiourea whether it acts as growth regulator or not in cotton plant under control and drought conditions. Discovered that externally applied thiourea as root growing medium is better for growth or not under control and drought conditions. And determined the changes in different attributes such as morpho-physiological and yield of cotton plant due to the thiourea under low water availability condition.

II. MATERIALS AND METHODS

2.1 Experimental layout

Two varieties (FH-142 and FH-942) of cotton (*Gossypium hirsutum* L.) were used in pot experiment to study the different aspects of morphological and physiological processes in response to externally applied thiourea as rooting medium. Two pot trials were carried out by using the soil filled plastic pots. To evaluate the effect of externally applied thiourea through root growing medium on cotton plant under low moisture condition, three level of thiourea (water, 10 mM and 20 mM) were used. Water stress (normal watering and 60% field capacity) was maintained after 49 days of sowing. Thiourea (water, 10 mM and 20 mM) were applied as root growing medium after two weeks of drought stress (after 63 days of sowing). Data for the following aspects were observed after three weeks of application of thiourea (after 83 days of sowing). The experiments were managed by using four replicates.

2.2 Growth attributes

Two plants were up rooted carefully, washed with distilled water and recorded their shoot fresh weight and shoot length after five weeks of maintaining the water deficit condition. The plants were oven-dried to get their shoot dry weight.

2.3 Photosynthetic pigments

Arnon (1949) method was used for recording the data of photosynthetic pigments (chlorophyll *a*, *b* and carotenoids). The extraction of 0.1 g fresh leaves was done in 80% acetone. The readings were recorded by using UV-visible spectrophotometer (IRMECO U2020) at different wavelength such as 480, 645 and 663 nm.

2.4 Gas exchange attributes

IRGA (infrared gas analyzer, Model LCA-4) is the instrument which was used to estimate the trace gasses by measuring the absorption of an emitted infrared light source through a certain air sample. It was used at 13.00-14.00 h to evaluate the data of gas exchange parameters like net CO_2 assimilation rate (A), stomatal conductance (g_s), transpiration rate (E) and sub-stomatal CO_2 concentration (C_i).

2.5 Mineral ions (Na^+, K^+ and Ca^{2+})

Flame photometer was used to evaluate the data of sodium (Na^+), calcium (Ca^{2+}) and potassium (K^+) ions. To evaluate the inorganic ions, Allen *et al.* (1986) procedure was used. To the 0.1 g shoot dry material, 2 ml of sulphuric acid were added, incubate the samples at room temperature for 24 hours. After 24 hours, the samples were added with 35% H_2O_2 and heated on hot plate (350ºC) for 50 min. The process was repeated until the samples become colorless. The volume of the samples was raised up to 50 ml by using distilled water.

2.6 Yield attributes

Different aspects of yield such as number and weight of bolls, number and weight of seeds and number of monopodial and sympodial branches were evaluated at maturity.

2.7 Statistical analysis

The experiment was laid down in a Completely Randomized Design (CRD) with four replicates. A three-way ANOVA was applied to observe the significance in data by following Snedecor and Cochran, (1980).

III. RESULTS

3.1 Growth Parameters

3.1.1 Shoot lenght

In recent research, low moisture stress condition (60% field capacity) considerably ($P \leq 0.001$) declined the length of shoot in cotton varieties (FH-142 and FH-942). Different level of thiourea applied through root growing medium did not show significant effect in both cotton varieties. Variety FH-942 performed better as compared to FH-142 under normal and stressed condition (Table 1; Figure 1).

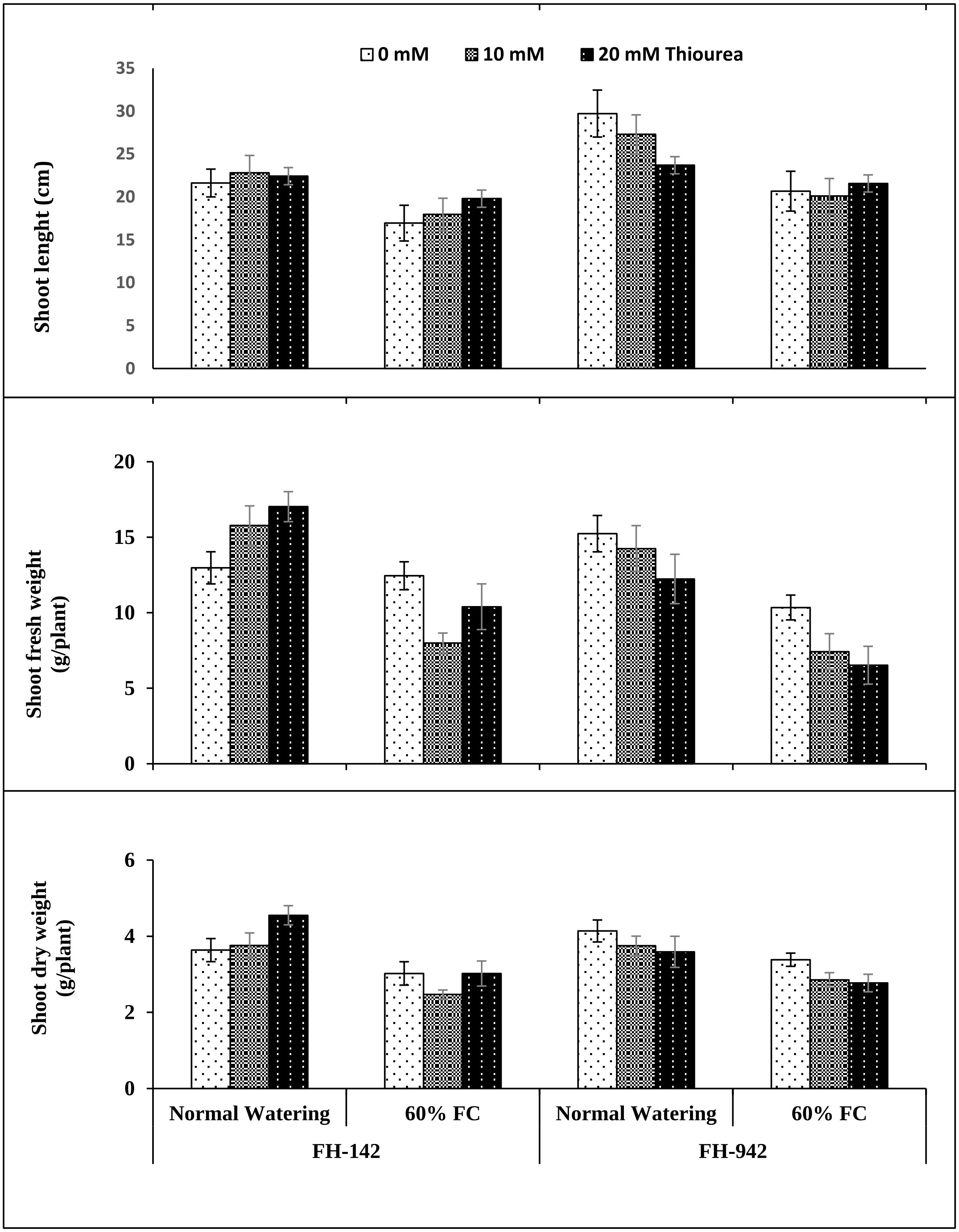
3.1.2 Shoot fresh and dry weight

Low availability of water condition markedly ($P \leq 0.001$) declined the fresh and dry weights of shoot in both (FH-142 and FH-942) cotton varieties. Externally applied thiourea as root growing medium markedly ($P \leq 0.05$) ($P \leq 0.001$) improved the fresh and dry weight of shoot in both varieties (FH-142 and FH-942) of cotton plant respectively. Among different level of thiourea, 20 mM enhanced the fresh and dry weight of shoot in FH-142. In our research, both varieties (FH-142 and FH-942) showed non-significant effect under normal and stress condition (Table 1; Figure 1).

Table 1. Mean squares of data for growth and gas exchange parameters from analyses of variance under normal watering and 60% field capacity in cotton (*Gossypium hirsutum* L.) plant when 63 days old plant were treated with different level of thiourea as rooting medium.

Source of variance	df	Shoot length	Shoot fresh weight	Shoot dry weight
Variety (V)	**1**	153.7252**	1.47ns	0.0285ns
Drought (D)	**1**	310.5918***	529.3408***	3.3443***
Thiourea (TU)	**2**	0.5443ns	49.6984*	5.0578***
V x D	**1**	12.9168ns	33.3333ns	0.0981ns
V x TU	**2**	19.4839ns	63.2151*	0.0922ns
D x TU	**2**	22.7743ns	2.7641ns	0.3969ns
V x D x TU	**2**	5.9981ns	54.7916*	0.1587ns
Error	**36**	18.7895	12.5098	0.2592
Source of variance	**df**	***A***	***E***	g_s
Variety (V)	**1**	2.8081**	0.0065ns	18.75ns
Drought (D)	**1**	13.1356***	0.0456*	1102.0833***
Thiourea (TU)	**2**	5.2569***	0.0238ns	400***
V x D	**1**	0.9047ns	0.0208ns	18.75ns
V x TU	**2**	0.7375ns	0.0397*	400***
D x TU	**2**	0.9282ns	0.0217ns	58.3333ns
V x D x TU	**2**	1.3673*	0.0224ns	25ns
Error	**36**	0.3106	0.0099	38.1944

*, ** and *** = significant at 0.05, 0.01, and 0.001 levels, respectively; ns = non-significant; df = degrees of freedom; A = net CO_2 assimilation rate; E = transpiration rate; g_s = stomatal conductance



0 mM
10 mM
20 mM Thiourea
Shoot lenght (cm)
35
30
25
20
15
10
5
0
Shoot fresh weight (g/plant)
20
15
10
5
0
Shoot dry weight (g/plant)
6
4
2
0
Normal Watering
60% FC
Normal Watering
60% FC
FH-142
FH-942

Figure 1. Growth traits under normal watering and 60% field capacity in cotton (*Gossypium hirsutum* L.) plant when 63 days old plant were treated with different level of thiourea as rooting medium.

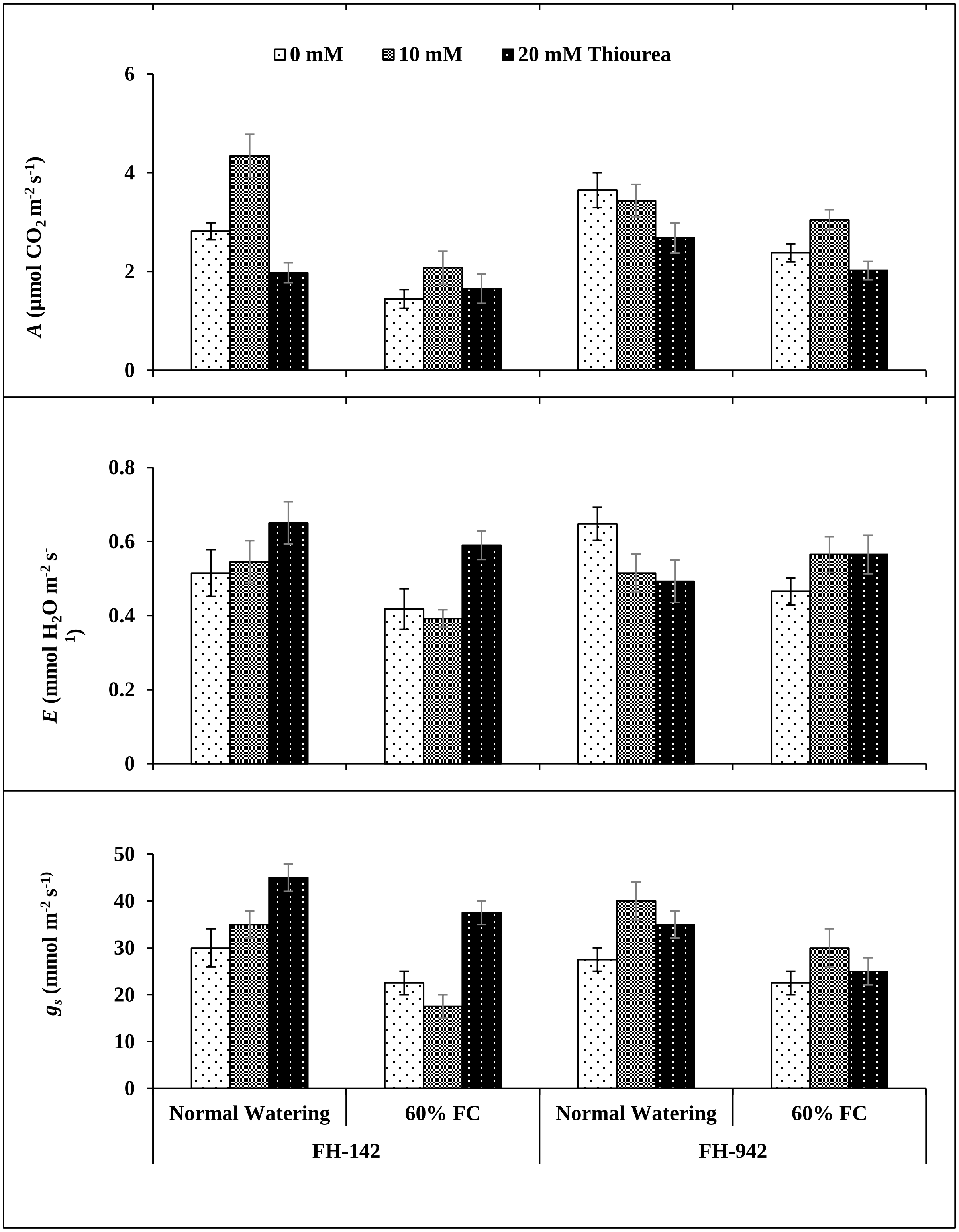


Figure 2. Gas exchange traits under normal watering and 60% field capacity in cotton (*Gossypium hirsutum* L.) plant when 63 days old plant were treated with different level of thiourea as rooting medium.

3.2 Gas exchange attributes

3.2.1 CO_2 assimilation rate

In our work, rate of CO_2 assimilation (*A*) considerably ($P \leq 0.001$) decreased due to the scarcity of water condition (60% field capacity) and externally applied thiourea through rooting medium considerably ($P \leq 0.001$) improved the rate of CO_2 assimilation (*A*) in both cotton varieties (FH-142 and FH-942). Of various levels of thiourea, 10 mM markedly improved the .Variety FH-942 perfomed better under normal and stress condition (Table 1; Figure 2).

3.2.2 Transpirational rate

In both varieties (FH-142 and FH-942) of cotton plant, low water availability condition markedly ($P \leq 0.05$) declined the rate of transpiration (*E*) while exogenously applied thiourea as rooting medium showed non- significant effect on both varieties of cotton (FH-142 and FH-942) plant. Both varieties (FH-142 and FH-942) showed non-significant effect under normal and 60% field capacity condition (Table 1; Figure 2).

3.2.3 Stomatal conductance

In both FH-142 and FH-942 cotton varieties, conductance of stomata (g_s) markedly ($P \leq 0.001$) declined under water scarcity condition (60% field capacity). Externally applied thiourea markedly ($P \leq 0.001$) amended the g_s in both FH-142 and FH-942 varieties of cotton plant under both normal watering and 60% field capacity. Both varieties showed non-significant effect under normal and stress condition. Thiourea (10 mM) improved the stomatal conductance in FH-942 and 20 mM in FH-142(Table 1; Figure 2).

3.2.4 Sub stomatal and relative sub-stomatal CO_2 concentration

In recent work, sub stomatal CO_2 concentration (C_i) and relative sub-stomatal CO_2 concentration (C_i/C_a) did not show significant effect in both varieties (FH-142 and FH-942) of cotton plant under low moisture stress condition. Externally applied thiourea showed non-significant effect on both varieties under normal and 60% field capacity. Both varieties FH-142 and FH-942 showed similar behavior under normal and stress condition (Table 2; Figure 3).

3.2.5 Water use efficiency

Low moisture stress considerably ($P \leq 0.001$) declined the *A/E* (water use efficiency) in both varieties (FH-142 and FH-942) of cotton plant in current study. Externally applied thiourea through rooting medium considerably ($P \leq 0.001$) adjusted the *A/E* (water use efficiency) under normal watering and 60% field capacity conditions in both FH-142 and FH-942 of cotton plant. Among different level of thiourea, 10 mM enhanced the water use efficiency more efficiently. FH-142 and FH-942 varieties did not show significant effect under normal and 60% field capacity condition (Table 2; Figure 3).

Table 2. Mean squares of data for gas exchange parameters from analyses of variance and chlorophyll pigments under normal watering and 60% field capacity in cotton (*Gossypium hirsutum* L.) plant when 63 days old plant were treated with different level of thiourea as rooting medium.

Source of variance	df	C_i	A/E	C_i/C_a
Variety (V)	**1**	3189.9102ns	2.9801ns	0.0257ns
Drought (D)	**1**	94.9218ns	16.8744***	0.000761ns
Thiourea (TU)	**2**	2513.8658ns	22.2912***	0.0202ns
V x D	**1**	1101.1252ns	0.6394ns	0.0088ns
V x TU	**2**	13905.101***	2.5247ns	0.1122***
D x TU	**2**	2297.9775ns	1.4341ns	0.0185ns
V x D x TU	**2**	1045.3958ns	5.3114**	0.0084ns
Error	**36**	1645.987	0.9453	0.0132
Source of variance	**df**	**Chl. *a***	**Chl. *B***	**Chl. *a/b***
Variety (V)	**1**	0.0021ns	0.000166ns	0.000283ns
Drought (D)	**1**	0.3727***	0.0207ns	0.1339ns
Thiourea (TU)	**2**	0.0926*	0.0802ns	0.0474ns
V x D	**1**	0.0031ns	0.000341ns	0.0029ns
V x TU	**2**	0.000181ns	0.0275ns	0.1698ns
D x TU	**2**	0.0281ns	0.0266ns	0.0335ns
V x D x TU	**2**	0.0349ns	0.09861*	0.3017ns
Error	**36**	0.0241	0.0294	0.0994

*, ** and *** = significant at 0.05, 0.01, and 0.001 levels, respectively; ns = non-significant; df = degrees of freedom; C_i = substomatal CO_2 concentration; A/E = water use efficiency; C_i/C_a = relative sub-stomatal CO_2 concentration

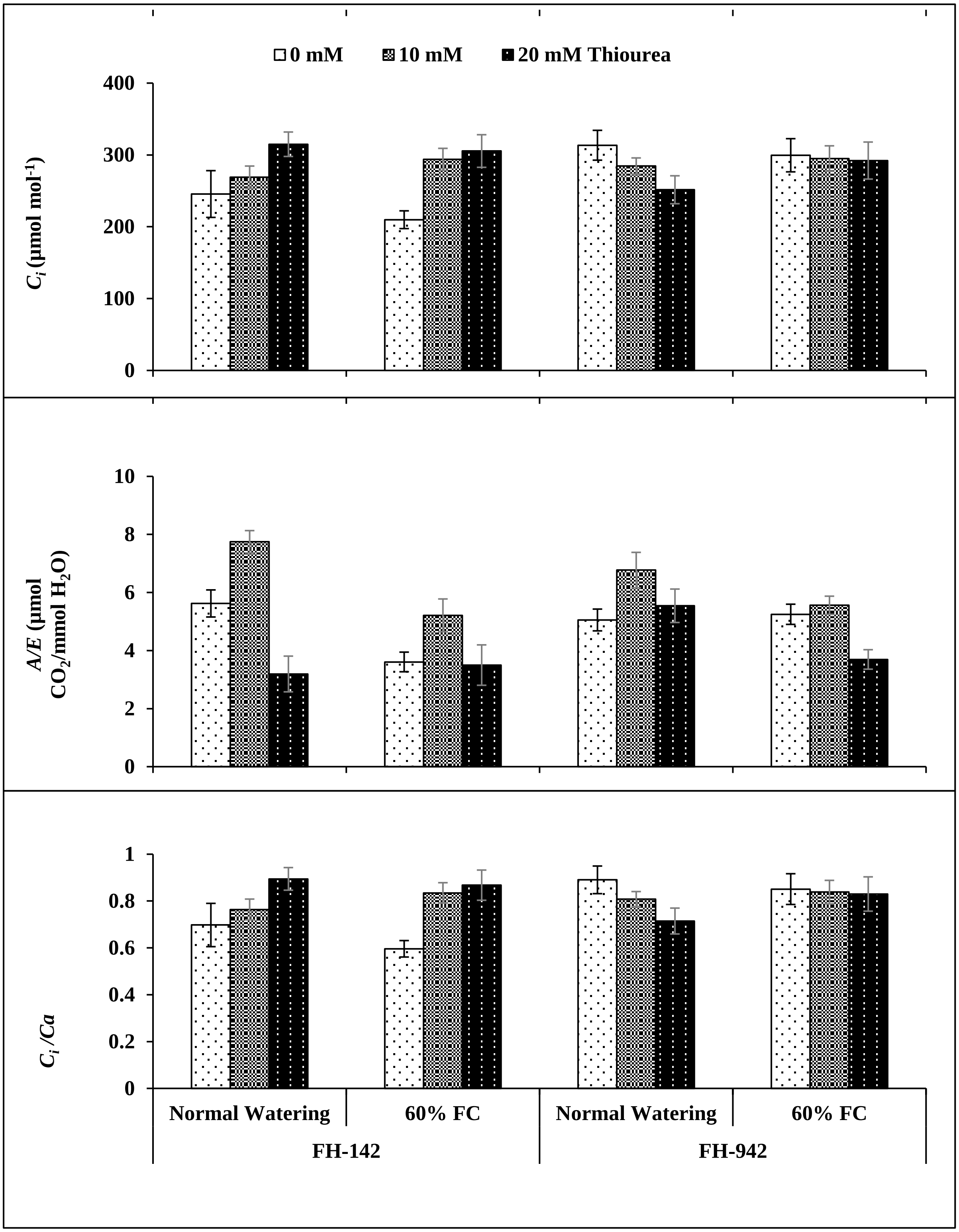


Figure 3. Gas exchange traits under normal watering and 60% field capacity in cotton (*Gossypium hirsutum* L.) plants when 63 days old plant were treated with different level of thiourea as rooting medium.

3.3 Photosynthetic pigments

3.3.1 Chlorophyll a contents

Shortage of water condition considerably ($P \leq 0.001$) decreased the contents of chlorophyll *a* in both cotton varieties (FH-142 and FH-942). Root growing medium application of thiourea considerably ($P \leq 0.05$) enhanced the content of chlorophyll *a* in both varieties of cotton plant under normal and 60% field capacity. Of various thiourea levels, 20 mM significantly improved the contents of chlorophyll *a*. Both varieties showed similar behavior under water and normal stress condition (Table 2; Figure 4).

3.3.2 Chlorophyll b contents and ratio of chlorophyll a/b

Low moisture stress condition and rooting medium application of thiourea did not showed significant effect on chlorophyll *b* content and ratio of chlorophyll *a/b* in both varieties under normal and 60% field capacity, both varieties FH-142 and FH-942 performed similar in both conditions (normal and water stressed) (Table 2; Figure 4).

3.3.3 Total chlorophyll contents

Both varieties FH-142 and FH-942 did not showed significant effect under water and stressed condition. Low water availability condition considerably ($P \leq 0.05$) declined the contents of total chlorophyll in both cotton varieties and externally applied thiourea as root growing medium markedly ($P \leq 0.05$) enhanced the total chlorophyll contents in both varieties under normal watering or 60% field capacity. Among different level of thiourea, 20 mM significantly improved the total chlorophyll contents (Table 3; Figure 5).

3.3.4 Carotenoid contents

Low water availability condition (60% field capacity) markedly decreased the content of carotenoid in both of cotton varieties (FH-142 and FH-942). Application of thiourea did not show considerable effect on both cotton varieties under normal and 60% field capacity condition. Varieties FH-142 and FH-942 performed similar behavior under both normal and water stress condition (Table 3; Figure 5).

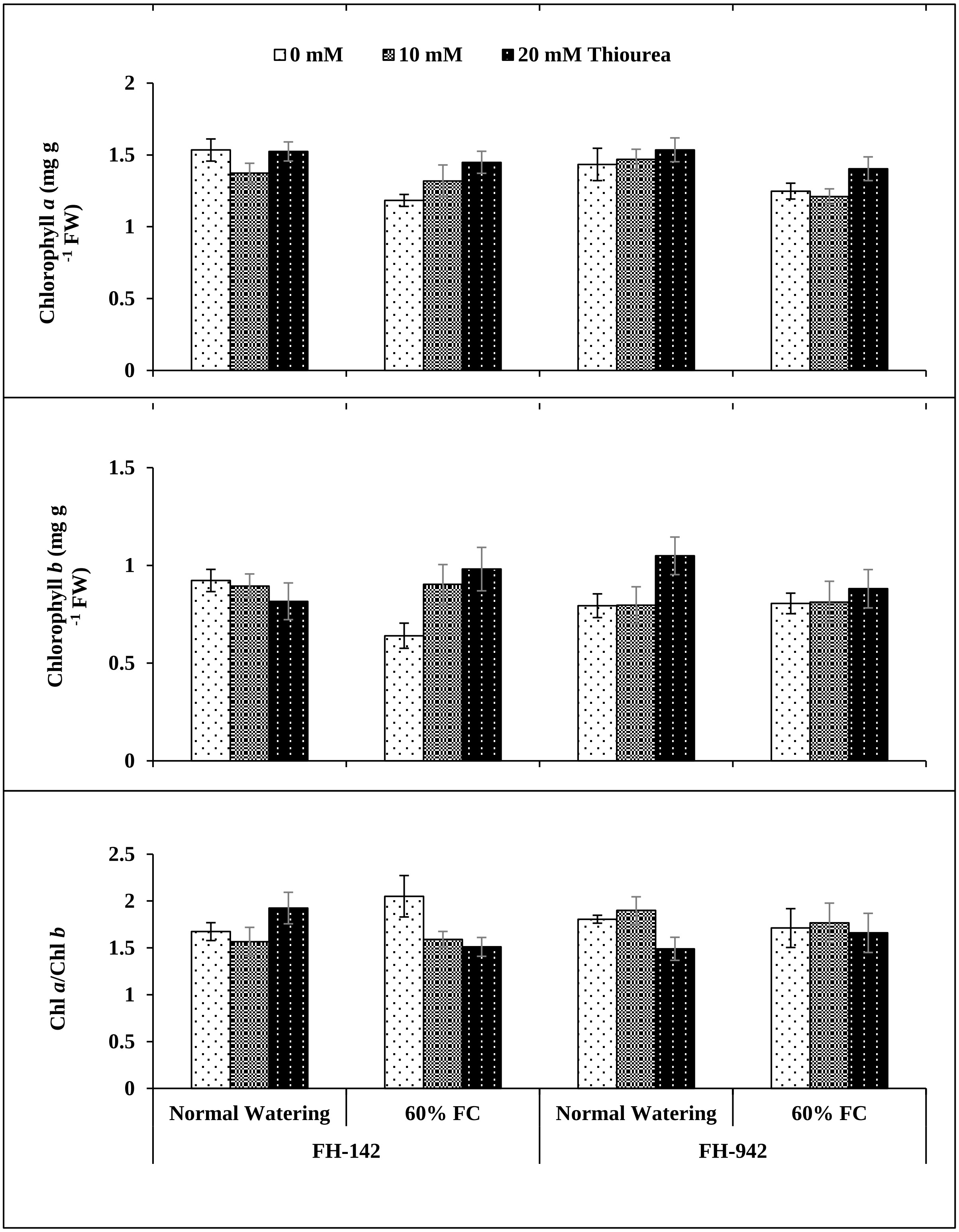
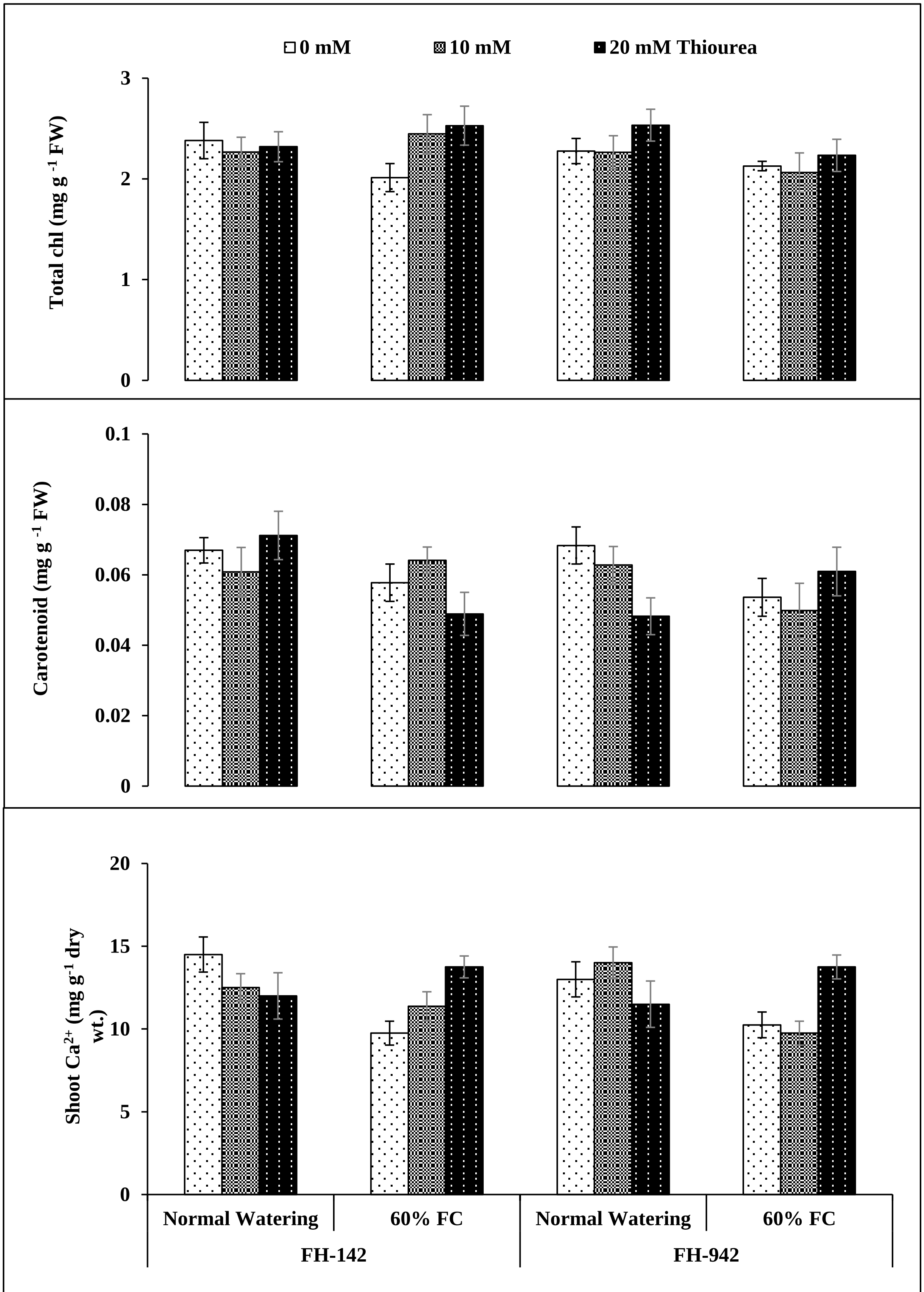


Figure 4. Chlrophyll pigments under normal watering and 60% field capacity in cotton (*Gossypium hirsutum* L.) plant when 63 days old plant were treated with different level of thiourea as rooting medium.

Table 3. Mean squares of data for chlorophyll pigments, mineral nutrients and yield parameters from analyses of variance under normal watering and 60% field capacity in cotton (*Gossypium hirsutum* L.) plant when 63 days old plant were treated with different level of thiourea as rooting medium

Source of variance	df	Total chl.	Carotenoid	Shoot Ca^{2+}
Variety (V)	1	0.0035ns	0.000227ns	0.8802ns
Drought (D)	1	0.5694*	0.000621*	26.2552*
Thiourea (TU)	2	0.3251*	0.0000757ns	3.9427ns
V x D	1	0.0055ns	0.0000585ns	0.1302ns
V x TU	2	0.0235ns	0.0000268ns	0.1927ns
D x TU	2	0.1051ns	0.0000682ns	37.4427***
V x D x TU	2	0.1984ns	0.000728**	6.9427ns
Error	36	0.0794	0.000135	3.7204
Source of variance	**df**	**Shoot K^+**	**Shoot Na^+**	**Number of bolls/plant**
Variety (V)	1	4.0833ns	1.5052ns	0.1875ns
Drought (D)	1	24.0833*	32.5052*	7.5208*
Thiourea (TU)	2	33.2343**	74.7708***	6.9375*
V x D	1	0.5208ns	7.9218ns	0.5208ns
V x TU	2	0.0364ns	0.6458ns	1.3125ns
D x TU	2	17.4114ns	5.64588ns	0.2708ns
V x D x TU	2	0.0052ns	2.4375ns	1.8958ns
Error	36	5.7013	5.3628	1.7986

*, ** and *** = significant at 0.05, 0.01, and 0.001 levels, respectively; ns = non-significant; df = degrees of freedom



0 mM
10 mM
20 mM Thiourea
Total chl (mg g -1 FW)
3
2
1
0
Carotenoid (mg g -1 FW)
0.1
0.08
0.06
0.04
0.02
0
Shoot Ca2+ (mg g-1 dry wt.)
20
15
10
5
0
Normal Watering
60% FC
Normal Watering
60% FC
FH-142
FH-942

Figure 5. Chlorophyll pigments and mineral nutrients under normal watering and 60% field capacity in cotton (*Gossypium hirsutum* L.) plant when 63 days old plant were treated

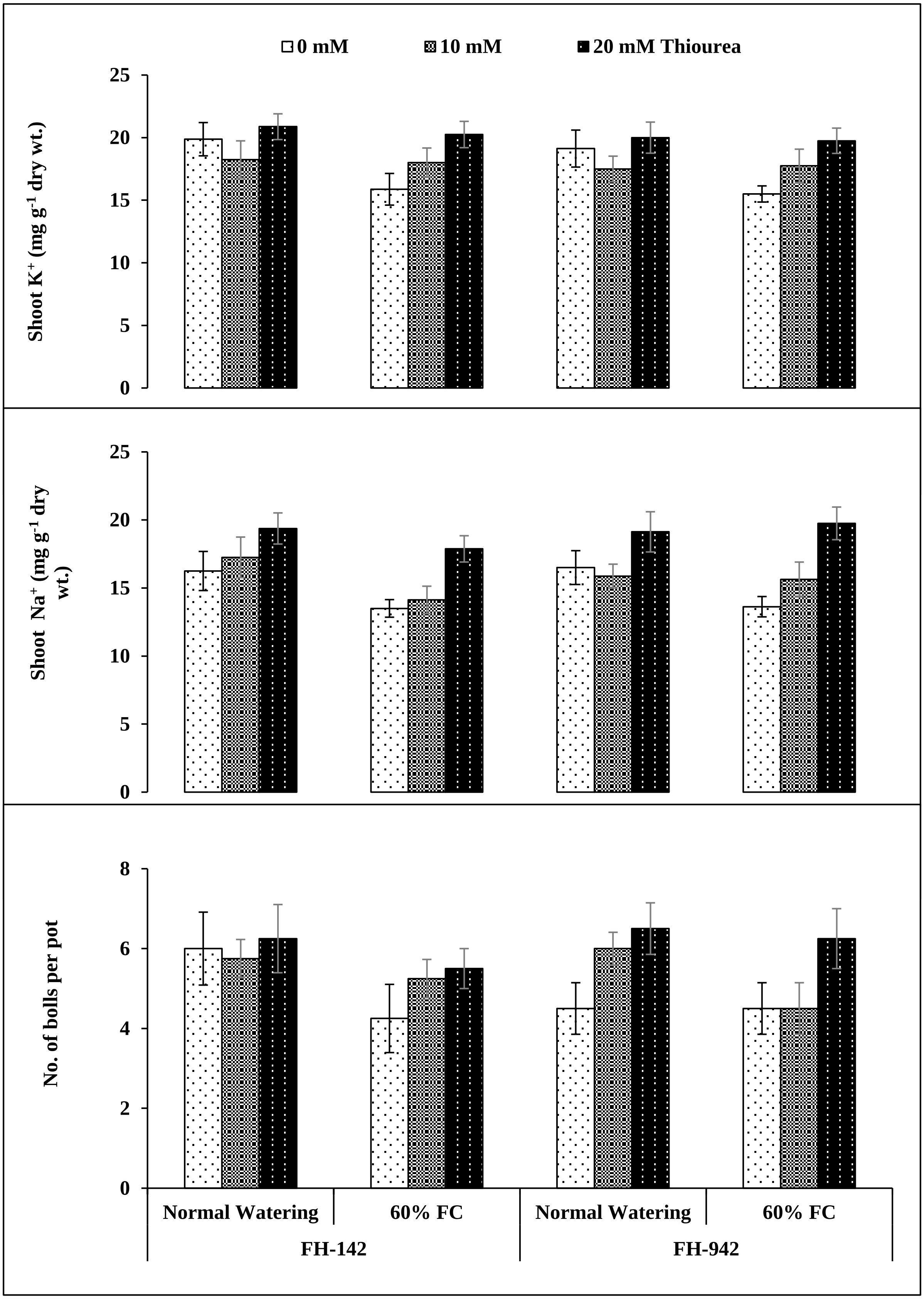


Figure 6. Mineral nutrients and yield traits under normal watering and 60% field capacity in cotton (*Gossypium hirsutum* L.) plant when 63 days old plant were treated with different level of thiourea as rooting medium.

Table 4. Mean squares of data for yield parameters from analyses of variance under normal watering and 60% field capacity in cotton (*Gossypium hirsutum* L.) plant when 63 days old plant were treated with different level of thiourea as rooting medium

Source of variance	df	Weight of bolls/plant	Number of seeds/plant	Weight of seeds/plant
Variety (V)	**1**	0.0833ns	8.3333ns	0.2133*
Drought (D)	**1**	0.9075***	52.0833**	1.47***
Thiourea (TU)	**2**	0.5575***	12.6458ns	0.3731***
V x D	**1**	0.0033ns	0.00000924ns	0.03ns
V x TU	**2**	0.0133ns	9.5208ns	0.0639ns
D x TU	**2**	0.03ns	13.1458ns	0.0493ns
V x D x TU	**2**	0.0358ns	0.4375ns	0.0193ns
Error	**36**	0.0518	5.1666	0.0418
Source of variance	**df**	**Number of monopodial branches/plant**	**Number of sympodial branches/plant**	
Variety (V)	**1**	0.75ns	0.3333ns	
Drought (D)	**1**	4.0833ns	12***	
Thiourea (TU)	**2**	2.1458ns	3.8125**	
V x D	**1**	1.3333ns	0.0833ns	
V x TU	**2**	0.8125ns	0.7708ns	
D x TU	**2**	0.5208ns	0.5625ns	
V x D x TU	**2**	1.0208ns	1.2708ns	
Error	**36**	1.0416	0.5555	

*, ** and *** = significant at 0.05, 0.01, and 0.001 levels, respectively; ns = non-significant; df = degrees of freedom

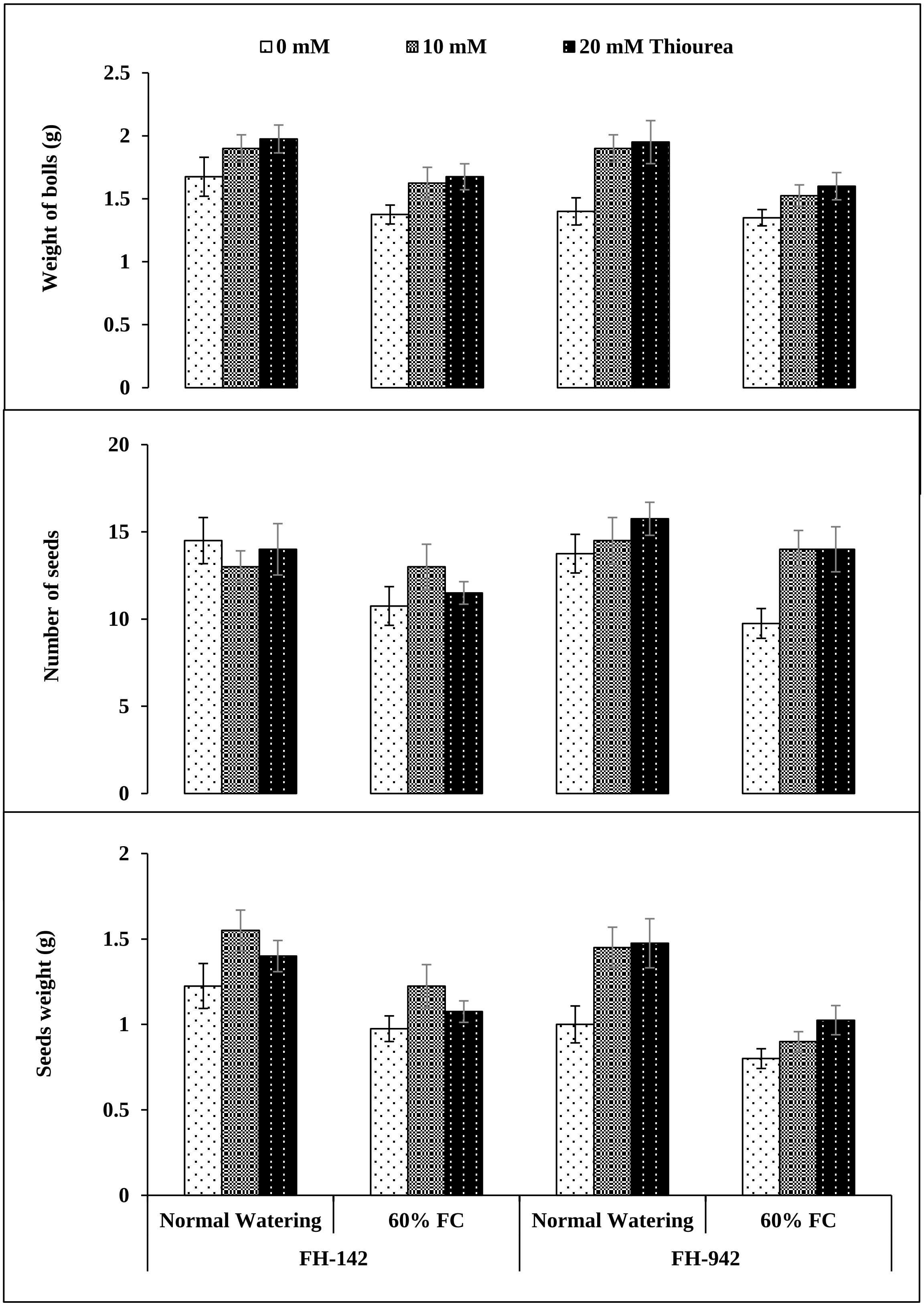


Figure 7. Mineral nutrients and yield traits under normal watering and 60% field capacity in cotton (*Gossypium hirsutum* L.) plant when 60 days old plant were treated with different level of thiourea as rooting medium.

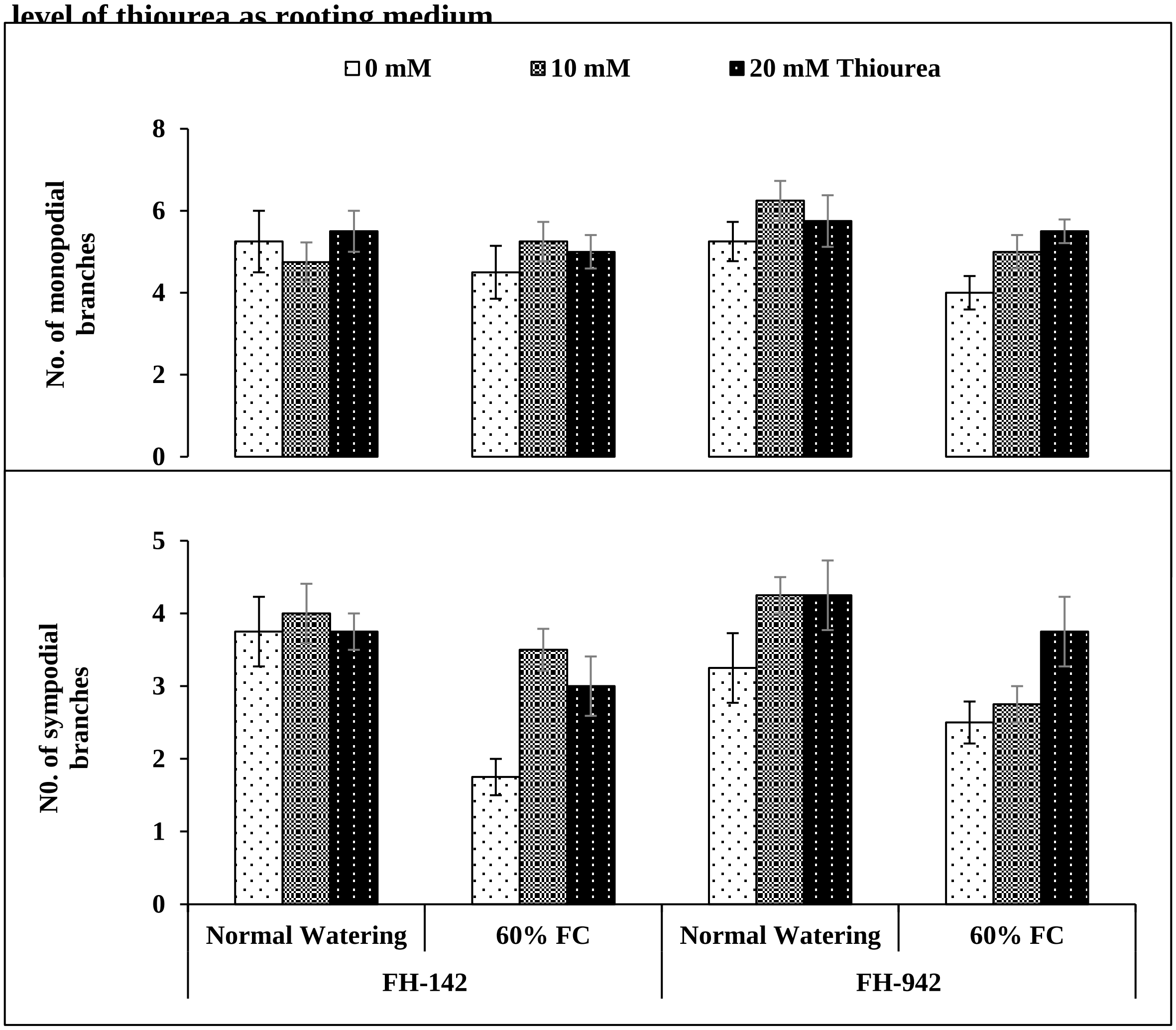


Figure 8. Mineral nutrients and yield traits under normal watering and 60% field capacity in cotton (*Gossypium hirsutum* L.) plant when 63 days old plant were treated with different level of thiourea as rooting medium.

3.4 Mineral ions

3.4.1 Shoot potassium and sodium ions

Both varieties FH-142 and FH-942 showed uniform behavior under normal and stress condition. Low moisture stress markedly ($P \leq 0.05$) lowered the shoot K^+ and Na^+ in both cotton varieties FH-142 and FH-942. Application of thiourea as root growing medium markedly ($P \leq 0.01$) ($P \leq 0.001$) amended the contents of potassium and sodium ions of shoot in both varieties (FH-142 and FH-942) of cotton plant under stress or non-stress conditions. At 20 mM thiourea shoot K^+ and Na^+ were high as compared to other levels (Table 3; Figure 6).

3.4.2 Shoot calcium ions

Water stress and externally applied thiourea as rooting medium showed non-significant effect on shoot calcium ions in both varieties FH-142 and FH-942 under normal watering and 60% field capacity. Both varieties showed uniform behavior under normal watering and water stress condition (Table 3; Figure 5).

3.5 Yield attributes

Low moisture stress condition markedly declined the yield of cotton plant in both varieties FH-142 and FH-942 such as weight and number of bolls per plant, number and weight of seeds per plant and number of sympodial branches. Root growing medium application of thiourea significantly amended the weight and number of bolls, weight of seeds and sympodial branches under normal and 60% field capacity. Both varieties performed similar behavior for number and weight of bolls per plant, number of seeds per plant and number of sympodial branches while both varieties showed significant effect on weight of seeds. Variety FH-142 performed better as compared to variety FH-942 (Table 3, 4; Figure 6, 7, 8).

The number of monopodial branches in both varieties FH-142 and FH-942 showed non-significant effect. Water stress and application of thiourea through root growing medium showed non-significant effect on number of seeds and monopodial branches under normal and stressed condition. Both varieties did not showed significant effects (Table 4; Figure 7, 8).

IV. DISCUSSION

4.1 Growth parameters

The different mechanism of plants disturbed such as photosynthesis, metabolism of nutrients, ions uptake and process of respiration which ultimately declined the growth parameters due to the lower water availability conditions (Li *et al*., 2011). In previous study, water scarcity condition markedly declined the shoot length in corn plant (Khan *et al*., 2015). Khayatnezhad *et al*. (2011) described in wheat plant that height of wheat plant significantly decreased due to low moisture stress. In corn plant, water stress declined the plant height (Khodarahmpour, 2011). Previous study favors the present study in which water stress significantly decreased the shoot length (Table 1; Figure 1). Low moisture availability condition repressed the process of enlargement and cell division and declined the cell turgidity due to shortage of water contents in soil which leads to the growth abnormalities (Pal *et al*., 2012). In our research, low water availability condition markedly declined the shoot dry and fresh weight in cotton plant (Table 1; Figure 1). Similar result found in previous study in which lower water availability condition markedly reduces the developmental aspects such as fresh and dry weight of shoot in sweet basil and lime basil (Khalid, 2006) and similar result also observed in maize plant (Hussain *et al*., 2018). In recent work, application of thiourea through rooting medium significantly amended the fresh and dry weight of shoot in cotton plant and did not showed significant effect on shoot length of cotton plant (Table 1; Figure 1). Previous study in maize plant favors the recent work in which externally applied thiourea markedly improved the developmental aspects such as fresh and dry weight of shoot (Perveen *et al*., 2013). Externally applied thiourea adjusted the turgidity of plants and activities of antioxidants which ultimately up-regulated the developmental aspects of plants (Hameed *et al*., 2013). Under water scarcity condition, thiourea maintain the turgidity of plants by improving the leaf RWC (relative water contents) and thus improved the developmental process of plants (Waqas *et al*., 2019). Related result also observed by Kaya *et al*. (2019) that externally applied thiourea also improved the growth by decreasing the electrolyte leakage due to membrane damage under low water conditions. Under water scarcity condition, application of thiourea as root growing medium improves the growth by enhancing the activities of enzymes which responsible for the uptake of growth enhancing substances (Abdelkader *et al*., 2012).

4.2 Gaseous exchange

Water scarcity condition markedly declined the different physiological parameters in different plants (Parveen *et al*., 2021). Different processes such as net CO_2 assimilation rate, transpiration rate, stomatal conductance, water use efficiency, sub-stomatal CO_2 concentration and C_i/C_a ratio in maize plant disturbed due to lower water availability condition (Anjum *et al*., 2011a). Low water availability decreased the process of photosynthesis in apple plant by decreasing the transpiration rate (Klamkowski and Treder, 2002). The findings are in accordance to recent work in which scarcity of water condition inhibited physiological process by decreasing the rate of CO_2 assimilation, rate of transpiration, conductance of stomata, and water use efficiency while sub stomatal CO_2 concentration and relative sub-stomatal CO_2 concentration remained unaffected in cotton plant (Table 1, 2; Figure 2, 3). Low moisture stress condition decreased the process of photosynthesis by decreasing the contents of water in soil which declined the turgidity of cell (Saeidi and Abdoli, 2015) and causes the closure of stomata. For the process of photosynthesis, adequate amount of CO_2 is required for normal functioning, but closure of stomata declined the uptake of CO_2 which inhibited the process of photosynthesis (Klamkowski *et al*., 2015). Physiological parameters also disturbed due to the production of oxygen radicals which damaged the photosystem (Basu *et al*., 2016). Application of thiourea amended the photosynthetic process by maintaining the opening of stomata in wheat plant (Wakchaure *et al*., 2016).The photosynthetic process enhanced by the exogenously applied thiourea (Baqer *et al*., 2020). Thiourea applied through root growing medium enhanced the process of photosynthesis by improving photosynthetic pigments in potato plant (Saleem *et al*., 2022). Similar result found in our research in which application of thiourea amended the assimilation of CO_2, conductance of stomata and water use efficiency which ultimately improved the physiological process (Table 1, 2; Figure 2, 3).

4.3 Photosynthetic pigments

In our work, different pigments of photosynthesis such as chlorophyll *a*, total chlorophyll and carotenoids contents markedly declined in cotton plant due to water scarcity condition (Table 2, 3; Figure 4, 5). Previous research favors our work in which water stress significantly lowered the contents of chlorophyll *a* in chickpea and beans (Lonbani and Arzani, 2011) and also reduced the contents of total chlorophyll in wheat plant (Sharifi and Mohammad Khani, 2016). Low water availability condition markedly lowered the photosynthetic pigments in potatoes cultivar (Saleem *et al*., 2022). Low moisture stress condition lowered the contents of chlorophyll by decreasing the light harvesting pigment protein complex (Ashraf and Harris, 2013) and by increasing the level of oxygen radicals (Zlatev and Lidon, 2012). In current study, different pigments of photosynthesis such as contents of chlorophyll *a* and total chlorophyll enhanced due to externally applied thiourea through rooting medium in cotton plant (Table 2, 3; Figure 4, 5). Similar result found in potato plant in which application of thiourea in root growing medium improved the chlorophyll contents (Saleem *et al*., 2022). Exogenously applied thiourea improved the chlorophyll contents by lowering the harmful effect of oxygen radicals on efficiency of photosystem II by lowering the lipid peroxidation in chickpea (Vineeth *et al*., 2016). Low moisture stress condition damaged the structure of chlorophyll and exogenously applied thiourea protects the chlorophyll structure by increasing the activities of protein kinases which ultimately leads to the improvement of growth and development (Jagetiya and Sharma, 2013).

4.4 Mineral ions

Water stress lowered the contents of ions in sugarcane (Waraich *et al*., 2011) and common bean (Ghanbari *et al*. 2013b). Shortage of water condition significantly lowered the potassium ion in sweet basil and lime basil (Khalid, 2006). Low moisture stress condition showed adverse effects in soybean by decreasing the potassium and calcium ions (Samarah *et al*., 2004). In our study, low water availability condition lowered the contents of shoot potassium and sodium ions while shoot calcium ions remain unchanged (Table 3; Figure 5, 6). Low moisture stress condition declined the ions contents by decreasing the enzyme activities which involved in the uptake of ions (Ashraf and Iram, 2005). Externally applied thiourea as rooting medium in our research increased the of potassium and sodium ions contents in cotton plant (Table 3; Figure 6). In coriander, externally applied thiourea increased the contents of different ions such as nitrogen, sulphur and potassium (Meena *et al*., 2015). The enzyme activities improved due to the externally applied thiourea which ultimately involved in the improvement of different ions contents (Choudhary *et al*., 2017).

4.5 Yield attributes

Water scarcity condition markedly declined the bolls number and weight per plant, seeds number and weight per plant and number of sympodial branches in both varieties of cotton plant (Table 3, 4; Figure 6, 7, 8). Similar results found in previous study such as in cotton (Pettigrew, 2004) and sugarcane (Vasantha *et al*., 2005) in which water stress lowered the yield. Low moisture stress condition lowered the yield in legumes by decreasing number of flowers and size of seed (Fang *et al*., 2010). In chickpea, water scarcity condition lowered the yield production up to 33%

(Kashiwagi *et al*., 2015). Water stress condition declined the yield production due to closure of stomata which inhibited the process of photosynthesis (Li *et al*., 2020). In our research, application of thiourea through rooting medium improved the number and weight of bolls, weight of seeds and sympodial branches in both cotton varieties (Table 3, 4; Figure 6, 7, 8). In previous research, application of thiourea enhanced the yield production in mung bean plant (Abdelkader *et al*., 2012) and removed the harmful effect of water scarcity condition in onion plant (Wakchaure *et al*., 2018). Exogenously applied thiourea which is nitrogen containing compound increased the production of different crops by breaking the bud dormancy (El-Keblawy, 2013) and seed dormancy (El-Keblawy and Gairola, 2017). Exogenously applied thiourea increased the process of photosynthesis by modulation of stomatal opening which in turn leads to the higher production of yield (Abdelkader *et al*., 2012). Exogenously applied thiourea increased the tuber weight by improving the developmental process (Baqer *et al*., 2020). Application of thiourea enhanced the rate of photosynthesis by up-regulating the Rubisco large subunit genes in response to low water availability (Vineeth *et al*., 2016). Under low water availability condition, oxygen free radicles are produced which decreases the crop production. Application of thiourea eliminated the harmful effects of oxygen free radicles and improved the developmental process of plants by maintaining the turgor and lowering the leakages of electrolyte by membrane which ultimately leads to the higher yield of different crops (Ratnakumar *et al*., 2016).

V. Conclusion

Water scarcity condition decreased the aspects of growth (shoot length, shoot fresh and dry weight), gaseous exchange parameters [CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance (g_s) and water use efficiency (A/E)], photosynthetic pigments (Chlorophyll *a*, total chlorophyll and carotenoid contents), shoot mineral ions (potassium, calcium and sodium ions) and yield attributes (number of bolls and seeds, weight of bolls and seeds and number of sympodial branches. Among different level of externally applied thiourea 10 mM as root growing medium significantly improved the rate of CO_2 assimilation (A) in both varieties, conductance of stomata (g_s) in FH-942, water use efficiency (A/E), weight of seeds and number of sympodial branches in FH-142. While application of 20 mM thiourea through rooting medium improved the growth aspects such as fresh and dry weight of shoot in FH-142, stomatal conductance in FH-142, Chlorophyll *a* contents, total chlorophyll contents, mineral ions (potassium and sodium), number and weight of bolls, weight of seeds in FH-942 and sympodial branches in FH-942. Among different levels of thiourea, 20 mM was better than other levels in enhancing the growth, photosynthetic pigments and yield attributes. Variety FH-942 performed better in increasing the shoot length and CO_2 assimilation rate (*A*) while variety FH-142 significantly increased the seeds weight under normal and stress condition.

Conflict of interest: All authors declare no conflict of interest.

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