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Effect of hydrogel on chlorophyll content and chlorophyll stability index of groundnut (*Arachis hypogaea* L.) under rainfed condition

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Abstract

A field experiment was conducted during *Kharif* 2018 at Zonal Agricultural and Horticultural Research Station, Hiriya, University of Agricultural and Horticultural Sciences, Shivamogga to study the effect of different levels of hydrogel, farm yard manure (FYM) and mulching on chlorophyll a, chlorophyll b, total chlorophyll content and chlorophyll stability index of groundnut under rainfed condition. The experiment was laid out in a Randomized Complete Block Design (RCBD) with ten treatments replicated thrice. The results revealed that, treatment with application of recommended dose of fertilizers (RDF) along with hydrogel @ 4 kg ha⁻¹ and FYM @ 10 t ha⁻¹ recorded significantly higher Chlorophyll 'a', Chlorophyll 'b', total chlorophyll content (before and after boiling) and higher chlorophyll stability index at different growth stages. However, significantly lower CSI was noticed in the treatment with application of RDF alone.

Keywords: Hydrogel, chlorophyll content, chlorophyll stability index, groundnut

Introduction

Groundnut (*Arachis hypogaea* L.) is an essential edible oil and food crop of the world. It is an annual and highly self-pollinated crop belongs to the family *leguminaceae* and subfamily *Papilionaceae*. It was introduced to India during the 18th century. It is a unique crop with attributes of both oil and proteins, consisting of 44 to 50 per cent of edible oil and 25 per cent of high-quality protein. Groundnut is an energy-rich crop and it needs fertile and well aerated soils. In recent years, the area under groundnut in dry lands is decreasing gradually due to erratic rainfall and low moisture availability at critical stages. Under these circumstances, we have to raise the crop by utilizing less amount of water to produce maximum yields. Hence, the combined use of hydrogel and nutrient management showed to be the best alternative to get higher pod yields with enhanced seed quality.

In arid and semi-arid regions with limited soil-water availability, hydrogel polymers can be used which enhance the water and nutrient use efficiency. When the root zone of the plant dries up, the hydrogel is able to retain water, plant nutrients and release it to the plant. Nowadays, soil moisture conservation is considered as one of the significant challenges for countries in arid and semi-arid regions. By 2030, global water demand is probable to be 50 per cent higher than today, resulting in water scarcity. At the same time the agricultural sector uses over 70 per cent of freshwater in most regions of the world.

Plant will experience severe water stress, if transpiration is very high and available water decreases near the root zone [1]. The use of farm yard manure along with hydrogel is very effective in reducing drought stress effect and also improves the yield and stability in agriculture production. Plant response to drought stress mainly depends on the intensity of water shortage. Plant shows short term or long term physiological response to the drought stress. Changes in leaf chlorophyll content and chlorophyll stability index are the short term reactions to stress [2].

Chlorophyll stability is a source indicator of plant resistance to environmental stresses [3, 4] reported that leaf chlorophyll content in soybean and bean reduced by drought stress. Leaf chlorophyll content is an important factor in determination of photosynthesis rates and dry

mater production [5]. Chlorophyll 'a' in wheat decreased in heading and 20 days after anthesis and chlorophyll 'b' decreased in heading stage only in drought stress condition [2-6] reported that chlorophyll content in wheat increased in flag leaf by drought stress in anthesis stage in comparison with non-stress condition. The result of some researches showed that cell wall was destroyed by increasing fat content in cell wall in drought stress [7, 8].

Material and Methods

A field experiment was conducted during *Kharif* 2018 at the Zonal Agricultural and Horticultural Research Station, Babbur farm, Hiriyyur, Karnataka. The experiment consisted of ten treatments and three replications. Treatments involved were FYM @ 10 t ha⁻¹ and mulching with pongamia green leaf at 4.0 t ha⁻¹ along with hydrogel (Figure 1) application with four doses *i.e.*, 1.0, 2.0, 3.0, 4.0 kg ha⁻¹. RCBD design was used in a test crop (Figure 2) groundnut (G2-52 variety) with spacing of 30 cm × 10 cm.



Fig 1: Hydrogel (dry)



Fig 2: General view of experimental plot at 60 DAS

Experimental details

Experimental Design: Randomized complete block design (RCBD)

No. of Treatments: Ten

No. of Replications: Three

Test crop: Groundnut

Variety: G2-52

Spacing : 30 cm × 10 cm

Gross plot size: 5 m × 4.5 m

Season: *Kharif*: 2018

Date of sowing: 14-07-2018

Date of harvesting: 03-12-2018

Treatment details

T₁: RDF (Control)

T₂: RDF + 1.0 kg hydrogel ha⁻¹

T₃: RDF + 2.0 kg hydrogel ha⁻¹

T₄: RDF + 3.0 kg hydrogel ha⁻¹

T₅: RDF + 4.0 kg hydrogel ha⁻¹

T₆: T₂+ 10 tons of FYM ha⁻¹

T₇: T₃+ 10 tons of FYM ha⁻¹

T₈: T₄+ 10 tons of FYM ha⁻¹

T₉: T₅+ 10 tons of FYM ha⁻¹

T₁₀: RDF + Mulching (Pongamia leaves @ 4 t ha⁻¹)

RDF: Recommended dose of Fertilizers - 50 per cent N + 100 per cent P and K as basal dose and 25 per cent N each at 25 and 40 DAS.

RDF = 25:50:25 kg N:P₂O₅:K₂O ha⁻¹ (rainfed)

During the investigation, various biochemical properties were analyzed at different crop growth stages.

Chlorophyll 'a', chlorophyll 'b' and total chlorophyll content

Three fully opened leaves from each of the five randomly selected plants were collected and brought to the laboratory in the polythene covers. Bottom and the top portion of the leaves were discarded. The middle portion was cut into small pieces and 1.0 g of this cut leaf material was homogenized with 10 ml of 80 per cent acetone in a mortar and pestle (Figure 3). The homogenized solution was decanted and filtered (figure 4) through a funnel using filter paper. Finally, the volume of the filtrate (figure 5) was made up to 25 ml in a volumetric flask using acetone. The absorbance was measured at 645, 652 and 663 nm for the determination of chlorophyll 'a', 'b' and total chlorophyll content, respectively by using spectrophotometer. Chlorophyll 'a', 'b' and total chlorophyll contents were calculated using the formulae given by [9] and expressed as mg g⁻¹ fresh weight.

$$\text{Chlorophyll 'a'} = 12.7(A_{663}) - 2.69(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll 'b'} = 22.9(A_{645}) - 2.69(A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{Total Chlorophyll} = 22.0(A_{645}) + 8.02(A_{663}) \times \frac{V}{1000 \times W}$$

Where,

A_{663} = Absorbance at wavelength 663 nm

A_{645} = Absorbance at wavelength 645 nm

V = volume of extract (ml)

W = Fresh weight of the sample (g)

Chlorophyll stability index (%)

Chlorophyll stability index (CSI) was calculated by dividing total chlorophyll content (after boiling: figure 7) by total chlorophyll content (before boiling: figure 6) and expressed in percentage.

Chlorophyll content (after boiling) was estimated by taking 1.0 g of fresh leaf sample in 100 ml beaker and 60 ml of distilled water was added and kept it on the hot plate. The contents with leaf sample and water was boiled for about 25 minutes. After cooling, leaf sample was removed and macerated with 10 ml of 80 per cent acetone in a mortar with pestle. Homogenized solution was filtered through a funnel using filter paper. Finally, filtered volume was made up to 25 ml in a volumetric flask using acetone. The absorbance of the extract was measured at 645, 652 and 663 nm for the determination of chlorophyll 'a', and 'b' and total chlorophyll content, respectively by using spectrophotometer. Chlorophyll stability index (CSI) was calculated and expressed as percentage as described by ⁹ by using the formula as given below.

$$\text{Chlorophyll stability index(\%)} = \frac{\text{Total chlorophyll content (after boiling)}}{\text{Total chlorophyll content (before boiling)}} \times 100$$

Statistical analysis

The experimental data was analysed by adopting Fisher's method of analysis of variance as outlined by ^[10]. The level of significance used in the 'F' test was at 5 per cent.

Results and Discussion

Application of recommended dose of NPK fertilizers along with hydrogel (4.0 kg ha⁻¹) and FYM (10 t ha⁻¹) recorded significantly higher biochemical parameters like chlorophyll a, chlorophyll b, total chlorophyll content, chlorophyll stability index in groundnut leaves at different growth stages. This is mainly due to the application of RDF along with hydrogel and FYM improves the biochemical parameters of groundnut like Chlorophyll a, b and total chlorophyll content and chlorophyll stability index. The application of hydrogel and manure increases the soil moisture by retaining it for a long period.

Hence, it reduces the moisture stress which leads to avoid cell wall rupture *i.e.*, CSI (Table 3) and enhances the synthesis of photosynthetic pigments and also increases the yield by increment in growth parameters at different growth stages.

Chlorophyll 'a', chlorophyll 'b', total chlorophyll content differed significantly at 30, 60 and 90 DAS among the various treatments (Table 1 and 2). The chlorophyll content was higher in 30 DAS compared to 60 and 90 DAS might be due to higher moisture retention by rainfall received and good climatic condition prevailed at vegetative stage. Chlorophyll a, chlorophyll b and total chlorophyll contents were higher in vegetative stage than reproductive stages. These results are similar with the findings of ^[11]. The synthesis of chlorophyll pigments were higher due to the supply of available nutrients under sufficient moisture conditions which lead to increased photosynthetic rate. The reduction in photosynthetic pigment contents at 60 and 90 DAS which might be due to the utilization of nutrients and photosynthetic pigments for grand growth activities *viz.*, flowering, pegging, nodulation and pod development ^[12].

The treatment with an application of only RDF recorded lower Chlorophyll 'a', 'b' and total chlorophyll contents and chlorophyll stability index due to moisture stress which lead to increased rate of cell division and rupture of cell wall led to oozing out of cell contents along with pigments which in turn affect the processes of thylakoid electron transport system, photophosphorylation and photosynthesis. This led to significant decrement in growth and yield of groundnut. Similar results were also reported by ^[13]. Several workers studied on chlorophyll pigments and their stability index as an indicator of drought avoidance mechanism ^[14].



Fig 3: Crushing of leaf samples with acetone



Fig 4: Filtration of crushed leaf samples by using filter paper



Fig 5: Variation in chlorophyll content of different samples



Fig 6: CSI estimation (before boiling)



Fig 7: CSI estimation (before boiling)

Table 1: Effect of hydrogel application on chlorophyll 'a', chlorophyll 'b' and total chlorophyll (before boiling) content of ground nut leaves at different growth stages

Treatments	Chlorophyll 'a'			Chlorophyll 'b'			Total chlorophyll		
	(mg g ⁻¹ fresh weight)								
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90DAS
T ₁ : RDF (Control)	0.899	0.947	0.515	0.384	0.406	0.252	1.284	1.353	0.767
T ₂ : RDF+ 1.0 kg hydrogel ha ⁻¹	1.012	1.075	0.611	0.434	0.434	0.299	1.446	1.509	0.910
T ₃ : RDF+ 2.0 kg hydrogel ha ⁻¹	1.066	1.188	0.664	0.457	0.457	0.325	1.523	1.645	0.989
T ₄ : RDF+ 3.0 kg hydrogel ha ⁻¹	1.129	1.219	0.671	0.484	0.489	0.329	1.613	1.708	1.000
T ₅ : RDF+ 4.0 kg hydrogel ha ⁻¹	1.206	1.247	0.703	0.497	0.521	0.344	1.703	1.768	1.047
T ₆ : T ₂ + 10 tons of FYM ha ⁻¹	1.236	1.489	0.894	0.524	0.638	0.438	1.760	2.127	1.332
T ₇ : T ₃ + 10 tons of FYM ha ⁻¹	1.365	1.573	0.954	0.581	0.674	0.467	1.946	2.247	1.421
T ₈ : T ₄ + 10 tons of FYM ha ⁻¹	1.413	1.591	1.004	0.605	0.669	0.492	2.018	2.260	1.496
T ₉ : T ₅ + 10 tons of FYM ha ⁻¹	1.429	1.628	1.109	0.612	0.741	0.543	2.041	2.369	1.652
T ₁₀ : RDF+ Mulching	1.164	1.245	0.745	0.495	0.534	0.365	1.659	1.779	1.110
S. Em (±)	0.076	0.049	0.074	0.038	0.34	0.037	0.098	0.082	0.116
C.D. at 5%	0.214	0.141	0.219	0.113	0.99	0.108	0.291	0.244	0.343

Note: RDF: Recommended dose of fertilizers

FYM: Farm yard manure

DAS: Days after sowing

Table 2: Effect of hydrogel application on chlorophyll 'a', chlorophyll 'b' and total chlorophyll (after boiling) content of ground nut leaves at different growth stages

Treatments	Chlorophyll 'a'			Chlorophyll 'b'			Total chlorophyll		
	← (mg g ⁻¹ fresh weight) →								
	30DAS	60DAS	90DAS	30DAS	60DAS	90DAS	30DAS	60DAS	90DAS
T ₁ : RDF(Control)	0.589	0.585	0.312	0.391	0.389	0.208	0.983	0.974	0.520
T ₂ : RDF+ 1.0 kg hydrogel ha ⁻¹	0.665	0.665	0.393	0.442	0.443	0.262	1.111	1.109	0.654
T ₃ : RDF+ 2.0 kg hydrogel ha ⁻¹	0.707	0.753	0.436	0.470	0.501	0.290	1.181	1.256	0.726
T ₄ : RDF+ 3.0 kg hydrogel ha ⁻¹	0.756	0.773	0.444	0.502	0.514	0.296	1.262	1.288	0.741
T ₅ : RDF+ 4.0 kg hydrogel ha ⁻¹	0.802	0.812	0.469	0.533	0.540	0.313	1.339	1.354	0.781
T ₆ : T ₂ + 10 tons of FYM ha ⁻¹	0.843	1.006	0.625	0.560	0.669	0.417	1.407	1.677	1.042
T ₇ : T ₃ + 10 tons of FYM ha ⁻¹	0.933	1.068	0.671	0.620	0.710	0.448	1.558	1.781	1.119
T ₈ : T ₄ + 10 tons of FYM ha ⁻¹	0.977	1.082	0.715	0.649	0.719	0.477	1.631	1.803	1.192
T ₉ : T ₅ + 10 tons of FYM ha ⁻¹	1.009	1.144	0.807	0.670	0.761	0.538	1.684	1.907	1.345
T ₁₀ : RDF+ Mulching	0.783	0.836	0.509	0.521	0.556	0.339	1.308	1.393	0.849
S. Em (±)	0.063	0.053	0.069	0.037	0.03	0.04	0.101	0.079	0.103
C.D. at 5%	0.187	0.156	0.202	0.101	0.082	0.107	0.279	0.232	0.305

Note: RDF: Recommended dose of fertilizers

DAS: Days after sowing

FYM: Farm yard manure

Table 3: Effect of hydrogel application on chlorophyll stability index of ground nut leaves at different growth stages

Treatments	30 DAS	60 DAS	90 DAS
	← (%) →		
T ₁ : RDF(Control)	76.56	72.02	67.71
T ₂ : RDF+ 1.0 kg hydrogel ha ⁻¹	76.82	73.50	71.89
T ₃ : RDF+ 2.0 kg hydrogel ha ⁻¹	77.54	76.33	73.37
T ₄ : RDF+ 3.0 kg hydrogel ha ⁻¹	78.23	75.44	74.07
T ₅ : RDF+ 4.0 kg hydrogel ha ⁻¹	78.61	76.58	74.61
T ₆ : T ₂ + 10 tons of FYM ha ⁻¹	79.94	78.86	78.23
T ₇ : T ₃ + 10 tons of FYM ha ⁻¹	80.07	79.24	78.72
T ₈ : T ₄ + 10 tons of FYM ha ⁻¹	80.84	79.79	79.65
T ₉ : T ₅ + 10 tons of FYM ha ⁻¹	82.53	80.51	81.37
T ₁₀ : RDF+ Mulching	78.84	78.33	76.45
S. Em (±)	0.89	0.60	1.06
C.D. at 5%	2.65	1.79	3.14

Note: RDF: Recommended dose of fertilizers

DAS: Days after sowing

FYM: Farm yard manure

Conclusion

Existence of variability in rainfall concerning annual as well as seasonal affected the plant growth due to unavailability of moisture during critical stages, especially in dry land areas. Therefore, hydrogel usage along with manure significantly increased leaf chlorophyll content and chlorophyll stability index of groundnut, which led to better crop growth and higher yield.

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