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EFFICACY OF SEAWEED (*KAPPAPHYCUS ALVAREZII*) SAP ON CHLOROPHYLL CONTENT OF WHEAT (*TRITICUM AESTIVAM* L.) FLAG LEAF

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ABSTRACT

Field experiments conducted at Birsa Agricultural University Kanke, Ranchi, Jharkhand, during *Rabi* of 2013-14 and 2014-15 to find out whether application of seaweed sap (*Kappaphycus alvarezii*) at six concentration (0.0, 2.5, 5.0. 7.5, 10.0 and 15.0%) either as foliar spray alone or in combination with seed soaking at different fertilizer level (100 and 50% RDF), can influence chlorophyll content of wheat, reveals that Chlorophyll (a, b and total) content in wheat increasing with crop age from 2 to 9 DAF and thereafter it gradually decreased, due to senescence of leaf. Crop fertilized with 100% RDF maintained higher chlorophyll (a, b and total) in the flag leaf than 50% RDF. Chlorophyll content increased with increasing concentration of seaweed sap up to 7.5% and thereafter it gradually decreased. Application of 7.5%K sap along with 100% RDF recorded higher chlorophyll content (a, b and total) than other combinations. Application of 7.5 % K sap along with 50 % RDF was able to maintain similar chlorophyll (a, b and total) content as that of wheat fertilized with100 % RDF alone, indicating that 7.5 % K sap was capable enough to compensate the 50 % fertilizer requirement of wheat crop. Comparison of chlorophyll content estimated by DMSO and Chlorophyll meter were equally good.

INTRODUCTION

Seaweed extracts are marine macro algae extract found in shallow coastal area and it contains many micro (Fe, Cu, Zn, Co, Mo, Mn, and Ni) and macro elements as well as it contains growth promoting hormones like auxins (IAA and IBA), Cytokinins, gibberellins (Crouch and Van staden, 1994) and metabolites like vitamins, fatty acids, organic matter and amino acids (Challen and Hemingway, 1965). Liquid extracts obtained from seaweeds are successfully used as foliar sprays for several crops (Bokil *et al*., 1974). The beneficial effect of different components of seaweed extract application may work synergistically at different concentrations, although the mode of action still remains unknown (Fornes *et al*., 2002). Application of seaweed sap increased yield as well as N, P and K content in grapevine (Turan and Köse, 2004) and in soybean (Mancuso *et al*., 2006 and Rathore *et al*., 2009). Gajewski *et al*., 2008 reported that application of Goteo (an organic mineral fertilizer which contains algae extract *Ascophyllum nodosum* with addition of phosphorus) increased yield, marketable heads as well as vitamin C content in Chinese cabbage compared to the untreated cabbage, where slightly higher nitrate content was noted. Application of seaweed extract significantly increased seed yield and pod weight as well as improved nutritional values of seeds, *i.e.*, protein and carbohydrates on pepper (Zodape *et al*., 2008 and Arthur *et al*., 2003) and mung bean (Zodape *et al*., 2010).

In cereal crops, the top-most leaf *i.e.*, flag leaf, is most important source of carbohydrate production during grain filling. It makes up approximately 75% of the effective leaf area that contributes to grain fill. Flag leaf is considered to be one of the greatest components in determining grain yield potential in most cereal crops (Hirota *et al*., 1990). The characteristics of flag leaf, particularly chlorophyll content have been considered to reflect photosynthetic activity. It absorb sunlight and uses its energy to synthesize carbohydrates, from CO_2 and water. Therefore, chlorophyll has been used as a sensitive indicator of plant physiological status. There are two. types of chlorophyll in plants *i.e.* chlorophyll 'a' and 'b', both of them work as photoreceptor in photosynthesis. Chlorophyll 'a' participate directly in the light absorption reaction of photosynthesis whereas chlorophyll 'b' differ from chlorophyll 'a', it is an accessory pigments and acts indirectly in photosynthesis by transferring the light which absorb chlorophyll 'a'. It has been reported that chlorophyll content had changed throughout the growing season of plants and many external sources also affect the chlorophyll content in the plant cells and begins to decline at the start of aging in plant leaf (Matile *et al*., 1988; Pulkrabek, 1998). Furthermore, changes in accumulation of chlorophyll in plants are affected by external growth conditions, and chlorophyll content reduces under stress environment (Masuda *et al.,* 2002). Some related studies showed that leaf chlorophyll content was positively correlated with photosynthetic capacity (Araus *et al*., 1997) and high chlorophyll content in leaves was considered as a favorable trait in crop production (Teng *et al*., 2004).

Seaweed sap contains betains and glycine, which are responsible for slowing down the degradation of leaf chlorophyll rather than increasing its content, can play significant role in maintaining greenness of the plants for a longer period and delay the loss of photosynthetic activity of isolated chloroplast during storage (Genard *et.al.* 1991). Keeping these points in view an investigation entitle "Efficacy of seaweed sap (*Kappaphycus alvarezii*) on chlorophyll content of wheat (*Triticum aestivam*) flag leaf" has been undertaken, to find out chlorophyll content in the wheat flag leaf applied with different concentration of seaweed sap along with 50 and 100% RDF and also to determine the relationship between chlorophyll content measured by DMSO methods and chlorophyll content index (CCI) measured by chlorophyll meter (Opti science CCM-200.

MATERIALS AND METHODS

Field experiments were conducted at Birsa Agricultural University Kanke, Ranchi, Jharkhand, India, during *Rabi* season of 2013-14 and 2014-15 to find out chlorophyll content of wheat flag leaf with application of seaweed sap (*Kappaphycus alvarezii*) at six concentration (0.0, 2.5, 5.0. 7.5, 10.0 and 15.0%) either as foliar spray alone or in combination with seed soaking at two fertilizer level *i.e.* 100 % Recommended dose of fertilizer *i.e.* 120 : 60 : 40 kg N, P_2O_5 and K_2O kg ha^{-1} and 50% RDF. Two set of experiments were conducted, one with spraying of seaweed sap only, thrice at 20 days interval starting from 25 DAS (days after sowing) ,whereas in second experiment seeds were soaked for overnight with respective sap concentration before sowing followed by spraying thrice at 20 days interval as in case of first experiment. Both the experiments were laid out in a randomized block design (RBD) with 12 treatments, replicated thrice. Crop was fertilized as per treatment through urea, diammonium phosphate and muriate of potash. Half of nitrogen, full dose of phosphorus and potassium was applied as basal and rest of nitrogen was top dressed equally in two splits at crown root initiation and maximum tillering stage. Chlorophyll content (a, b and total) of wheat flag leaf were estimated at an weekly interval starting from 2 to 23 Days after flowering (DAF). During first and second year of experimentation chlorophyll content was estimated by Di-methyle sulphoxide method, (Hiscox and Israelstam, 1979), and during second year, Opti Science CCM-200 hand held chlorophyll content meter (Apogee 2006,Richardson *et al.*2002) was also used to record chlorophyll content index (CCI). From each treatment of both the experiments, four leaves were selected for the assessment of chlorophyll content. Chlorophyll content meter CCM-200 works on the basis of absorbance determination at two wave length i.e. 653 and 931 nano meter (nm) and calculate CCI value which is the ratio of Transmittance at 931nm to the Transmittance at 653nm,that is proportionate to the amount of chlorophyll content in the sample. CCI value is relative chlorophyll content, absolute chlorophyll content per unit area is not computed however it can be estimated by establishing a relationship between CCI value measured by Chlorophyll meter and chlorophyll content measured by Di-methyl sulphoxide solvent analysis (DMSO) method. Immediately after measuring of CCI for assessment of chlorophyll concentration, leaf disk (area sampled by instrument opti science CCM-200) were chopped and 100 mg sample was placed in a 7 mL DMSO, the chlorophyll was extracted by incubating at 65ºC for one hour until chopped leaf disk were color less. The extracted liquid was transferred to a graduated tube and made up to total volume of 10 ml by pure DMSO and 3 mL sample of extract was transferred to a cuvett tube. The absorbance of the DMSO chlorophyll extractants and blank (pure DMSO) were measured at 645 and 663 nm, using a spectrophotometer and finally chlorophyll a, b and total were calculated by following equations developed by Arnon's, 1949.

Chl. a (mg g^{-1}) = $[12.7 (A_{663})-2.69 (A_{645})] \times V / 1000 \times W$............(1)

Chl. b (mg g^{-1}) = $[22.9 (A_{645}) -4.68 (A_{663})] \times V / 1000 \times W$..........(2)

Total chl. (mg g^{-1}) = $[20.2 (A_{645}) + 8.02 (A_{663})] \times V / 1000 \times W$ ....(3)

Where, 'A' is the absorbance value measured by spectrophotometer at specific wave length (645 and 663 nm); 'V' is the Volume of solvent (10 ml) and 'W' is the Fresh weight of chopped leaf (100 mg).

Standard curve preparation

We used the 50 flag leaf samples of wheat having wide range of chlorophyll content as possible from very pale to dark green colour.CCI values of these leaves were recorded with chlorophyll content meter CCM-200.These leaves are then chopped and chlorophyll was extracted by DMSO methods followed by the determination of chlorophyll absorbance with 645 and 663 nm and polynomial regression equation was developed (Fig 1.) between CCI value and chlorophyll a, b and total chlorophyll content determined by DMSO method along with their regression coefficient (r^2). Afterwards these equations are used to estimate the chlorophyll content from observed CCI value measured by chlorophyll content meter (CCM-200). Comparison of chlorophyll content estimated by both these methodologies i.e. DMSO and Chlorophyll meter were done by using χ^2 test (Gomez and Gomez, 1984). Treatment comparison of chlorophyll content measured at different days after flowering were made by using Analysis of Variance technique suggested by (Gomez and Gomez, 1984

RESULTS AND DISCUSSION

Comparison of chlorophyll measured by DMSO and chlorophyll meter CCM-200

Standard curve was developed (Fig. 1) using the polynomial relationship between CCI value measured by Opti Science CCM-200 chlorophyll meter and chlorophyll content (a, b and total) estimated by DMSO extraction method. Regression coefficient value (r^2) of more than 0.6 in all chlorophyll a, b and total indicated that there is a strong relationship between these two methodologies. In both the experiment chlorophyll content estimated directly by DMSO extraction (Table 1 and 2) and by Opti Science CCM-200 chlorophyll meter CCI value converted to absolute chlorophyll (a, b and total) content (Table 3 and 4), were compared for their authenticity by using goodness of fit χ^2 test. Significance level of the χ^2 test indicated that chlorophyll content measured by both these methodologies is equally good. However, the absolute values of chlorophyll (a, b and total) content measured by chlorophyll meter were higher than the chlorophyll content measured by DMSO method.

Table 1: Effect of spraying of sea weed (*Kappaphycusalvarezii*) sap concentration and fertilizer level on chlorophyll content (mg g^{-1}) of wheat flag leaf measured by DMSO method (pooled data of 2 yr)

K Sap Concentration (%)	2 DAF Chlorophyll a			Chlorophyll b			Total chlorophyll		
	100%RDF	50%RDF	Mean	100%RDF	50%RDF	Mean	100%RDF	50%RDF	Mean
0.0 water spray	10.75	8.40	9.58	16.93	13.76	15.34	27.68	22.16	24.92
2.5	11.17	9.76	10.46	21.00	15.85	18.42	32.17	25.60	28.89
5 .0	11.33	9.98	10.65	21.42	16.17	18.80	32.76	26.15	29.45
7.5	11.88	10.39	11.14	25.78	19.89	22.84	37.67	30.29	33.98
10.0	10.89	9.84	10.37	21.50	17.72	19.61	32.39	27.56	29.98
15.0	10.80	9.41	10.11	19.56	16.05	17.81	30.37	25.45	27.91
Mean	11.14	9.63		21.03	16.57		32.17	26.20	
	Sap Conc.	Fertilizer	Sap X Fert	Sap Conc.	Fertilizer	Sap X Fert	Sap Conc.	Fertilizer	SapXFert
SEm(±)	0.81	0.57	0.16	0.68	0.48	0.14	0.99	0.70	0.20
CD(5%)	NS	NS	0.48	2.00	1.42	0.41	2.90	2.05	0.59
Sap Conc. (%)	9 DAF								
0.0water spray	12.21	9.04	10.62	21.63	18.88	20.25	33.84	27.91	30.88
2.5	13.44	9.41	11.42	22.67	17.45	20.06	36.11	26.86	31.49
5 .0	13.68	10.50	12.09	22.99	21.19	22.09	36.67	31.69	34.18
7.5	14.18	12.07	13.12	24.10	21.68	22.89	38.28	33.74	36.01
10.0	13.30	11.70	12.50	22.69	19.56	21.12	35.99	31.26	33.62
15.0	12.74	10.04	11.39	22.37	20.04	21.21	35.11	30.08	32.60
Mean	13.26	10.46		22.74	19.80		36.00	30.26	
	Sap Conc.	Fertilizer	Sap X Fert	Sap Conc.	Fertilizer	Sap X Fert	Sap Conc.	Fertilizer	Sap X Fert
SEm(±)	0.53	0.37	0.11	1.06	0.75	0.22	1.29	0.91	0.26
CD(5%)	1.54	1.09	0.31	NS)	2.20	0.64	3.78	2.67	0.77
Sap Conc. (%)	16 DAF								
0.0water spray	8.89	7.49	8.19	10.81	8.30	9.55	19.70	15.79	17.74
2.5	9.27	8.20	8.74	12.07	9.55	10.81	21.35	17.75	19.55
5 .0	9.87	8.45	9.16	13.72	10.15	11.94	23.59	18.60	21.09
7.5	11.46	8.54	10.00	15.16	11.81	13.49	26.62	20.36	23.49
10.0	9.64	8.08	8.86	13.60	10.03	11.82	23.24	18.11	20.68
15.0	8.99	7.97	8.48	13.23	8.58	10.91	22.22	16.55	19.39
Mean	9.69	8.12		13.10	9.74		22.79	17.86	
	Sap Conc.	Fertilizer	Sap X Fert	Sap Conc.	Fertilizer	Sap X Fert	Sap Conc.	Fertilizer	Sap X Fert
SEm(±)	0.57	0.40	0.06	0.63	0.45	0.13	1.00	0.71	0.21
CD(5%)	1.66 (NS)	1.17	0.18	1.85	1.31	0.38	2.95	2.08	0.60
Sap Conc. (%)	23 DAF								
0.0water spray	5.76	4.14	4.95	9.34	8.70	9.02	15.10	12.83	13.96
2.5	6.24	5.17	5.71	9.96	9.39	9.68	16.20	14.57	15.38
5 .0	6.70	5.50	6.10	10.60	9.64	10.12	17.30	15.14	16.22
7.5	7.37	5.71	6.54	13.13	10.23	11.68	20.50	15.94	18.22
10.0	6.26	5.15	5.71	11.85	10.03	10.94	18.11	15.18	16.64
15.0	6.01	4.61	5.31	10.45	8.91	9.68	16.47	13.51	14.99
Mean	6.39	5.05		10.89	9.48		17.28	14.53	
	Sap Conc.	Fertilizer	Sap X Fert	Sap Conc.	Fertilizer	Sap X Fert	Sap Conc.	Fertilizer	Sap X Fert
SEm(±)	0.26	0.18	0.05	0.54	0.38	0.11	0.57	0.40	0.12
CD(5%)	0.76	0.54	0.16	1.58	1.12	0.32	1.67	1.18	0.34

Efficacy of seaweed sap and fertilizer on chlorophyll content of wheat

Effect of fertilizer on chlorophyll content

Chlorophyll a, b and total content of wheat flag leaf increased with increasing crop age from 2 to 9 DAF and thereafter it gradually decreased (Table 1 2, 3& 4) due to senescence of flag leaf. In the first experiment where seaweed (*Kappaphycus alvarezii*) sap was sprayed only, wheat fertilized with 100% RDF recorded higher chlorophyll (a, b and total) content in the flag leaf than crop fertilized with 50% RDF (Table 1 & 3). Applications of 100% RDF recorded 11.14, 21.03 and 32.17 mg g^{-1} chlorophyll a, b and total at 2 DAF by DMSO method, whereas, By CCI method corresponding values are 11.75,20.03 and 31.78 mg g^{-1} respectively, which was 18.48, 26.91 and 22.78% higher than that of crop fertilized with 50% RDF in DMSO method . Similarly chlorophyll content (a, b and total) at 9 DAF were 13.26, 22.74 and 36.0 mg g^{-1} respectively which was 26.77, 14.84 and 18.96% higher than 50% RDF by DMSO method and corresponding values estimated by CCI methods are 11.93,20.67 and 32.60 mg g^{-1} respectively. At 16 DAF, crop fertilized with 100% RDF recorded 9.69, 13.10 and 22.79 mg g^{-1} chlorophyll a, b and total respectively which was 19.33, 34.49 and 27.60 % higher than 50% RDF by DMSO method, whereas CCI method estimated 11.89,21.13 and 33.02 mg g^{-1} chlorophyll a, b and total respectively. Whereas at 23 DAF, crop fertilized with 100% RDF recorded 6.39, 10.89 and 17.28 mg g^{-1} chlorophyll

Table 2: Effect of seed soaking and spraying with sea weed (*Kappaphycusalvarezii*) sap concentration and fertilizer level on chlorophyll content (mg g-1) of wheat flag leaf measured by DMSO method (pooled data of 2 year)

K Sap Concentration (%)	2 DAF Chlorophyll a			Chlorophyll b			Total chlorophyll		
	100%RDF	50%RDF	Mean	100%RDF	50%RDF	Mean	100%RDF	50%RDF	Mean
0.0 water spray	11.17	8.82	10.00	17.79	14.70	16.25	28.95	23.53	26.24
2.5	11.70	9.44	10.57	19.84	17.95	18.89	31.54	27.38	29.46
5 .0	12.63	9.98	11.31	21.16	20.29	20.73	33.79	30.27	32.03
7.5	15.29	11.28	13.28	25.76	22.76	24.26	41.05	34.03	37.54
10.0	12.12	10.18	11.15	21.17	20.81	20.99	33.29	30.99	32.14
15.0	11.77	9.12	10.44	19.67	18.26	18.96	31.44	27.37	29.41
Mean	12.45	9.80		20.90	19.13		33.34	28.93	
	Sap Conc.	Fertilizer	Sap X Fert	Sap Conc.	Fertilizer	Sap X Fert	Sap Conc.	Fertilizer	Sap X Fert
SEm(±)	0.55	0.39	0.11	0.91	0.65	0.19	1.05	0.74	0.21
CD(5%)	1.60	1.13	0.33	2.68	1.90 (NS)	0.55	3.09	2.18	0.63
Sap Conc. (%)	9 DAF								
0.0water spray	12.34	10.18	11.26	14.21	11.68	12.95	26.55	21.86	24.21
2.5	13.24	10.78	12.01	14.92	12.91	13.92	28.16	23.70	25.93
5 .0	13.64	11.23	12.43	16.25	13.35	14.80	29.88	24.58	27.23
7.5	13.86	11.96	12.91	17.74	13.80	15.77	31.61	25.76	28.68
10.0	13.08	11.05	12.06	14.84	12.60	13.72	27.96	23.65	25.80
15.0	12.67	10.47	11.57	14.78	12.00	13.39	27.40	22.48	24.94
Mean	13.14	10.95		15.46	12.72		28.59	23.67	
	Sap Conc.	Fertilizer	Sap X Fert	Sap Conc.	Fertilizer	Sap X Fert	Sap Conc.	Fertilizer	Sap X Fert
SEm(±)	0.56	0.39	0.11	0.83	0.59	0.17	1.11	0.79	0.23
CD(5%)	1.63	1.16	0.33	2.43	1.72	0.50	3.27	2.31	0.67
Sap Conc. (%)	16 DAF								
0.0water spray	10.10	8.43	9.27	18.79	11.67	15.23	28.90	20.10	24.50
2.5	10.35	9.40	9.87	20.78	15.31	18.05	31.13	24.71	27.92
5 .0	11.14	9.49	10.32	21.20	16.45	18.83	32.34	25.95	29.14
7.5	11.53	9.97	10.75	23.88	17.21	20.54	35.41	27.18	31.29
10.0	10.73	9.08	9.90	20.64	16.04	18.34	31.37	25.12	28.25
15.0	10.49	8.72	9.61	19.65	15.42	17.53	30.14	24.13	27.14
Mean	10.72	9.18		20.82	15.35		31.55	24.53	
	Sap Conc.	Fertilizer	Sap X Fert	Sap Conc.	Fertilizer	Sap X Fert	Sap Conc.	Fertilizer	Sap X Fert
SEm(±)	0.37	0.26	0.07	0.76	0.54	0.16	0.75	0.53	0.15
CD(5%)	1.07	0.76	0.22	2.24	1.58	0.46	2.20	1.56	0.45
Sap Conc. (%)	23 DAF								
0.0 water spray	5.68	4.16	4.92	18.00	15.06	16.53	23.68	19.21	21.45
2.5	6.63	4.17	5.40	19.70	16.75	18.23	26.33	20.92	23.63
5 .0	6.97	5.24	6.10	21.21	16.95	19.08	28.19	22.19	25.19
7.5	8.02	5.54	6.78	23.56	17.28	20.42	31.58	22.82	27.20
10.0	6.79	4.80	5.79	19.44	16.82	18.13	26.23	21.62	23.92
15.0	6.22	4.74	5.48	19.00	15.75	17.38	25.21	20.49	22.85
Mean	6.72	4.77		20.15	16.44		26.87	21.21	
	Sap Conc.	Fertilizer	Sap X Fert	Sap Conc.	Fertilizer	Sap X Fert	Sap Conc.	Fertilizer	Sap X Fert
SEm(±)	0.29	0.20	0.06	0.74	0.52	0.15	0.87	0.62	0.18
CD(5%)	0.85	0.60	0.17	2.17	1.53	0.44	2.56	1.81	0.52

a, b and total respectively by DMSO method which was 26.53, 14.87 and 18.92% higher than 50% RDF, whereas by CCI method it was recorded 11.61,17 and 28.61 mg g^{-1} chlorophyll a, b and total respectively.

Similar trend was also observed in the second experiment where seeds were soaked in sea weed sap before sowing followed by spraying in the standing crop as in the first experiment (Table 2 and 4). At 2 DAF, crop fertilized with 100% RDF recorded 12.5, 20.9 and 33.3 mg g^{-1}chllorophyll a, b and total respectively by DMSO method, which was 27.0, 9.25 and 15.2 % higher than that of crop fertilized with 50% RDF and by CCI method it was estimated 12.61,21.21 and 33.81 mg g^{-1}chllorophyll a, b and total respectively. Similarly at 9 days after flowering, applications of 100% RDF recorded higher chlorophyll a, b and total *i.e.*13.14, 15.46 and 28.59 mg g^{-1} respectively by DMSO method which was 20.0, 21.54 and 20.79 % higher than crop fertilized with 50% RDF, whereas by CCI method estimated 12.02,22.35 and 34.37 mg g^{-1} chlorophyll a, b and total respectively. At 16 DAF, crop fertilized with 100% RDF recorded 10.72, 20.82 and 31.55 mg g^{-1} chlorophyll a, b and total respectively by DMSO method, which was 9.05, 35.63 and 28.61 % higher than crop fertilized with 50% RDF, whereas by CCI method it was 11.60,20.71 and 32.30 mg g^{-1} chlorophyll a, b and total respectively. At 23 DAF, chlorophyll content with 100% RDF recorded 6.72, 20.15 and 26.87 mg g^{-1} chlorophyll a, b and total respectively by DMSO method, which was 40.88, 22.56 and 26.68 % higher than 50% RDF, whereas by CCI method it was 10.78,16.35 and 27.13 mg g^{-1} chlorophyll a, b and total respectively .

Table 3: Effect of spraying of sea weed (*Kappaphycusalvarezii*) sap concentration and fertilizer level on chlorophyll content(mg g^{-1}) of wheat flag leaf measured by chlorophyll meter during 2014-15

K Sap Concentration (%)	2 DAF CCI								
	Chlorophyll a			Chlorophyll b			Total		
	100%RDF	50%RDF	Mean	100%RDF	50%RDF	Mean	100%RDF	50%RDF	Mean
0.0 (Water spray)	11.49	11.38	11.44	19.72	19.43	19.57	31.21	30.81	31.01
2.5	11.72	11.41	11.57	19.92	19.44	19.68	31.64	30.85	31.25
5.0	11.97	11.53	11.75	20.32	19.49	19.90	32.28	31.02	31.65
7.5	12.23	11.60	11.91	20.74	19.70	20.22	32.97	31.30	32.13
10.0	11.55	11.58	11.56	19.75	19.56	19.65	31.29	31.14	31.22
15.0	11.52	11.41	11.47	19.73	19.41	19.57	31.25	30.82	31.04
Mean	11.75	11.49		20.03	19.50		31.78	30.99	
	Sap Conc.	Fertilizer	SapXFert	Sap Conc.	Fertilizer	SapXFert	Sap Conc.	Fertilizer	SapX Fert
SEm(±)	0.40	0.28	0.08	0.60	0.43	0.12	0.99	0.70	0.20
CD(5%)	1.18	0.83	0.24	1.76	1.25	0.36	2.91	2.05	0.59
Sap Conc.(%)	9 DAF CCI								
0.0 (Water spray)	11.81	11.41	11.61	19.35	15.93	17.64	31.16	27.34	29.25
2.5	11.92	11.44	11.68	21.86	18.12	19.99	33.79	29.56	31.67
5.0	12.05	11.65	11.85	21.88	18.80	20.34	33.93	30.45	32.19
7.5	12.08	11.89	11.98	22.28	19.20	20.74	34.36	31.09	32.72
10.0	11.90	11.73	11.81	19.83	18.56	19.20	31.73	30.29	31.01
15.0	11.82	11.67	11.75	18.84	17.71	18.28	30.66	29.39	30.02
Mean	11.93	11.63		20.67	18.05		32.60	29.69	
	Sap Conc.	Fertilizer	SapXFert	Sap Conc.	Fertilizer	SapXFert	Sap conc.	Fertilizer	SapXFert
SEm(±)	0.81	0.57	0.16	1.71	1.21	0.35	1.76	1.25	0.36
CD(5%)	2.37	1.67	0.48	5.03	3.56	1.03	5.17	3.66	1.06
SapConc.(%)	16 DAF CCI								
0.0 (Water spray)	11.74	10.86	11.30	20.95	19.45	20.20	32.69	30.31	31.50
2.5	11.76	11.37	11.57	20.97	19.56	20.27	32.73	30.94	31.83
5.0	11.84	11.47	11.65	21.04	20.39	20.72	32.88	31.86	32.37
7.5	12.06	11.55	11.80	21.36	20.56	20.96	33.42	32.11	32.76
10.0	12.06	10.99	11.52	21.27	20.67	20.97	33.33	31.66	32.50
15.0	11.92	10.81	11.36	21.17	19.73	20.45	33.09	30.54	31.81
Mean	11.89	11.17		21.13	20.06		33.02	31.24	
	Sap Conc.	Fertilizer	SapXFert	Sap conc.	Fertilizer	SapXFert	Sap conc	Fertilizer	SapXFert
SEm(±)	0.89	0.63	0.18	0.84	0.59	0.17	0.31	0.22	0.06
CD(5%)	2.62	1.85	0.53	2.46	1.74	0.50	0.90	0.64	0.18
SapConc.(%)	23 DAF CCI								
0.0 (Water spray)	11.44	10.52	10.98	16.87	16.15	16.51	28.31	26.67	27.49
2.5	11.47	11.03	11.25	16.90	16.81	16.85	28.37	27.83	28.10
5.0	11.56	11.34	11.45	17.08	16.81	16.94	28.64	28.15	28.39
7.5	11.82	11.39	11.60	17.15	16.83	16.99	28.97	28.22	28.59
10.0	11.71	11.36	11.53	17.04	16.55	16.80	28.75	27.91	28.33
15.0	11.66	10.87	11.27	16.96	16.45	16.70	28.62	27.32	27.97
Mean	11.61	11.08	10.98	17.00	16.60		28.61	27.68	
	Sap Conc.	Fertilizer	SapXFert	Sap Conc.	Fertilizer	SapXFert	Sap Conc.	Fertilizer	SapXFert
SEm(±)	0.25	0.18	0.05	0.178	0.13	0.04	0.44	0.31	0.09
CD(5%)	0.75	0.53	0.15	0.522	0.37	0.11	1.29	0.91	0.26

Higher chlorophyll content in flag leaf of wheat was recorded with 100% fertilizer application in both the experiment clearly indicates that flag leaf of fully fertilized crops are more capable of synthesizing CO_2 in to carbohydrate and there by producing more grain yield (Hirota *et al.,* 1990). Further it has been reported that chlorophyll content had changed throughout the growing season of plants, and chlorophyll content of plants begins to decline at the start of aging in plant leaf (Matile *et al.*, 1988; Pulkrabek, 1998). Araus *et al.* (1997) also reported that leaf chlorophyll content was positively correlated with photosynthetic capacity and high chlorophyll content in leaves was considered as a favorable trait in crop production (Teng *et al.,* 2004).

Effect of seaweed sap on chlorophyll content

Increasing seaweed sap concentration up to 7.5% gradually increased chlorophyll content (a, b and total) in flag leaf and thereafter it gradually decreased in both the expreriment (Table 1, 2, 3 & 4).

In the first experiment, where K sap was sprayed only, application of 7.5% K sap maintained significantly higher chlorophyll (a, b and total) in the flag leaf than other concentration (Table 1 & 3). At 2 DAF, crop sprayed with 7.5% K sap recorded 11.14, 22.84 and 33.98 mg g^{-1} chlorophyll a, b and total respectively, by DMSO method which was 16.28, 48.89 and 36.35% higher than water spray (Table 1), whereas corresponding CCI values are 11.91, 20.22 and 32.13 mg g^{-1}chlorophyll a, b and total (Table 3). At 9 DAF, crop sprayed with 7.5 % K sap recorded 13.12, 22.89 and

Table 4: Effect of seed soaking and spraying with sea weed (*Kappaphycus alvarezii*) sap concentration and fertilizer level on chlorophyll content (mg g-1) of wheat flag leaf measured by chlorophyll meter (2014-15)

K Sap Concentration (%)	2 DAF CCI Chlorophyll a 100%RDF	50%RDF	Mean	Chlorophyll b 100%RDF	50%RDF	Mean	Total 100%RDF	50%RDF	Mean
0.0 (Water spray)	12.47	11.90	12.19	21.05	20.27	20.66	33.52	32.16	32.84
2.5	12.61	11.95	12.28	21.21	20.39	20.80	33.82	32.34	33.08
5.0	12.64	12.42	12.53	21.25	20.88	21.06	33.89	33.29	33.59
7.5	12.83	12.50	12.66	21.39	21.15	21.27	34.22	33.65	33.93
10.0	12.62	12.30	12.46	21.28	20.83	21.06	33.90	33.14	33.52
15.0	12.48	12.08	12.28	21.05	20.58	20.81	33.53	32.66	33.10
Mean	12.61	12.19		21.21	20.68		33.81	32.87	
	Sap Conc.	Fertilizer	SapXFert	Sap Conc.	Fertilizer	SapXFert	Sap Conc.	Fertilizer	SapXFert
SEm(±)	0.09	0.07	0.02	0.16	0.11	0.03	0.30	0.21	0.06
CD(5%)	0.27	0.19	0.06	0.46	0.33	0.09	0.89	0.63	0.18
Sap Conc.(%)	9 DAF CCI								
0.0 (Water spray)	11.83	11.60	11.71	20.96	17.42	19.19	32.79	29.02	30.91
2.5	11.86	11.76	11.81	22.23	19.66	20.95	34.09	31.42	32.75
5.0	11.97	11.93	11.95	23.51	19.78	21.65	35.49	31.71	33.60
7.5	12.23	11.98	12.11	24.10	20.44	22.27	36.34	32.42	34.38
10.0	12.15	11.80	11.97	21.98	20.21	21.10	34.13	32.01	33.07
15.0	12.08	11.65	11.86	21.31	20.11	20.71	33.39	31.76	32.58
Mean	12.02	11.79		22.35	19.61		34.37	31.39	
	Sap Conc.	Fertilizer	SapXFert	Sap Conc.	Fertilizer	SapXFert	Sap Conc.	Fertilizer	SapXFert
SEm(±)	0.80	0.57	0.16	1.21	0.86	0.25	1.23	0.87	0.25
CD(5%)	2.35	1.66	0.48	3.55	2.51	0.72	3.62	2.56	0.74
SapConc.(%)	16 DAF CCI								
0.0 (Water spray)	11.48	10.50	10.99	20.53	19.03	19.78	32.01	29.52	30.77
2.5	11.49	10.55	11.02	20.55	19.65	20.10	32.04	30.21	31.12
5.0	11.52	11.42	11.47	20.94	20.41	20.67	32.46	31.82	32.14
7.5	11.79	11.42	11.61	20.94	20.45	20.70	32.73	31.88	32.30
10.0	11.75	11.08	11.42	20.68	19.86	20.27	32.43	30.94	31.68
15.0	11.55	10.94	11.25	20.60	18.97	19.78	32.15	29.90	31.03
Mean	11.60	10.98		20.71	19.73		32.30	30.71	
	Sap Conc.	Fertilizer	SapXFert	Sap Conc.	Fertilizer	SapXFert	Sap Conc.	Fertilizer	SapXFert
SEm(±)	0.88	0.62	0.18	0.70	0.49	0.14	0.58	0.41	0.12
CD(5%)	2.59	1.83	0.53	2.05	1.44	0.42	1.69	1.20	0.35
SapConc.(%)	23 DAF CCI								
0.0 (Water spray)	10.32	8.61	9.47	15.98	14.53	15.26	26.31	23.14	24.73
2.5	10.53	9.01	9.77	16.18	14.88	15.53	26.72	23.90	25.31
5.0	10.60	9.62	10.11	16.62	15.34	15.98	27.21	24.97	26.09
7.5	11.31	10.17	10.74	16.78	15.88	16.33	28.09	26.05	27.07
10.0	11.10	9.93	10.51	16.39	15.67	16.03	27.49	25.61	26.55
15.0	10.82	9.18	10.00	16.18	15.04	15.61	27.00	24.22	25.61
Mean	10.78	9.42		16.35	15.23		27.13	24.65	
	Sap Conc.	Fertilizer	SapXFert	Sap Conc.	Fertilizer	SapXFert	Sap Conc.	Fertilizer	SapXFert
SEm(±)	0.41	0.29	0.08	0.33	0.24	0.06	0.72	0.51	0.15
CD(5%)	1.22	0.86	0.25	0.98	0.69	0.199	2.10	1.49	0.43

36.01 mg g-1 chlorophyll a, b and total respectively by DMSO method which was 23.54, 13.03 and 16.61 % higher than control, whereas by CCI method estimated 11.98, 20.74 and 32.72 mg g-1 chlorophyll a, b and total respectively. At 16 DAF, crop sprayed with 7.5 % K sap recorded 10.00, 13.49 and 23.49 mg g-1 chlorophyll a, b and total respectively by DMSO method, which was 22.10**,** 41.25 and 32.41 % higher than control, whereas by CCI method estimated 11.8, 20.96 and 32.76 mg g-1 chlorophyll a, b and total respectively (Table 3). At 23 DAF, by DMSO method when crop sprayed with 7.5 % K sap recorded 6.54, 11.68 and 18.22 mg g-1 chlorophyll a, b and total respectively which was 32.12, 29.49 and 30.51% higher than control, whereas by CCI methods estimated 11.6,16.99 and 28.59 mg g-1 chlorophyll a, b and total respectively.

Similar trend was also observed in the second experiment where seeds were soaked with different concentration of seaweed sap before sowing followed by spraying in the standing crop as in first experiment (Table 2 & 4). At 2 DAF, crop sprayed with 7.5% K sap recorded 13.28, 24.26 and 37.54 mg g-1 chlorophyll a, b and total respectively by DMSO method which was 32.8, 49.29 and 43.06 % higher than control (Table 2), whereas by CCI method recorded 12.66, 21.27 and 33.93 mg g-1 chlorophyll a, b and total respectively (Table 4). Similarly at 9 DAF, crop sprayed with 7.5 % seaweed sap recorded 12.91, 15.77and 28.68 mg g-1 chlorophyll a, b and total respectively by DMSO method which was 14.65, 21.77 and 18.46 % higher than control whereas by CCI method

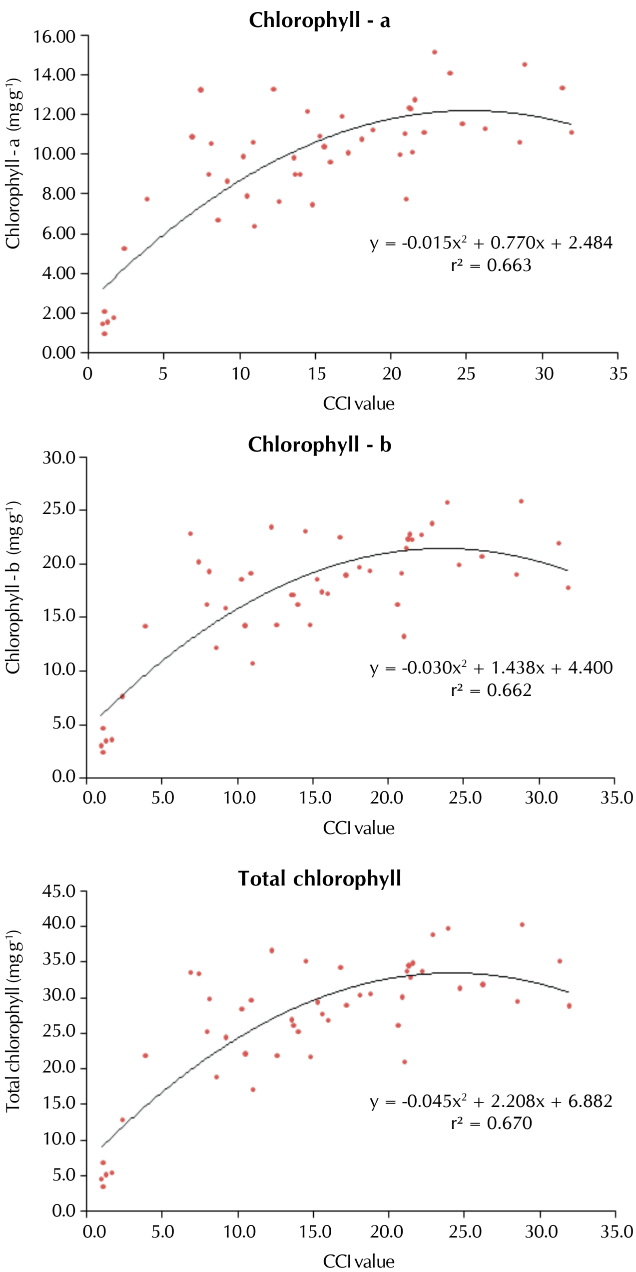

Figure 1: Scatter plots indicating the polynomial relationship between chlorophyll (a, b and total) content (mg g^{-1}) of flag leaf of wheat measured by DMSO method and chlorophyll content index (CCI) value measured by Opti-Science CCM-200 hand held chlorophyll meter

estimated 12.11, 22.27 and 34.38 mg g^{-1} chlorophyll a, b and total respectively. At 16 DAF, crop sprayed with 7.5 % K sap recorded 10.75, 20.54 and 31.29 mg g^{-1} chlorophyll a, b and total respectively by DMSO method and was 15.96, 34.86 and 27.71 % higher than control whereas corresponding CCI method estimated values are 11.61,20.70 and 32.30 mg g^{-1} chlorophyll a, b and total respectively. At 23 DAF, crop sprayed with 7.5 % K sap recorded 6.78, 20.42 and 27.20 mg g^{-1} chlorophyll a, b and total respectively by DMSO method, which was 37.80, 23.53 and 26.80 % higher than control whereas by CCI method estimated values are 10.74,16.33 and 27.07 mg g^{-1} chlorophyll a, b and total respectively .

C. Effect of seaweed sap and fertilizer level on chlorophyll content

In the first experiment, maximum chlorophyll (a, b and total) content of wheat flag leaf was recorded, with spraying 7.5 % K sap along with 100% RDF throughout grain filling period *i.e.* 11.8 , 25.78 & 37.67 ; 14.18 ,24.10 & 38.28 ; 11.46 , 15.16 & 26.62 and 7.37, 13.13 & 25.50 mg g^{-1} chlorophyll a, b and total at 2, 9, 16 and 23 DAF respectively by DMSO method (Table 1), whereas by CCI method corresponding values are 12.23, 20.74, 32.97; 12.08, 22.28, 34.36; 12.06, 21.36, 33.42 and 11.82,11.6, 16.99 mg g^{-1} chlorophyll a, b and total at 2, 9, 16 and 23 DAF respectively (Table 3).

Whereas in the second experiment soaking of seed with 7.5% K sap before sowing followed by its spraying on standing crop at 100 % RDF recorded 15.29, 25.76 &41.05; 13.86, 17.74 & 31.61; 11.53, 23.88 & 35.41 and 8.02, 23.56 & 31.58 mg g^{-1} chlorophyll a, b and total at 2, 9, 16 and 23 DAF respectively by DMSO method (Table 2) whereas by CCI method corresponding values are 12.83 ,21.39 ,34.22; 12.23 , 24.10, 36.34 ;11.79, 20.94, 32.73 ; and 11.10, 16.39, 27.49 mg g^{-1} chlorophyll a, b and total at 2, 9, 16 and 23 DAF respectively (Table 4). In both the experiment, spraying of 7.5 % seaweed sap along with 50 % RDF was able to maintain similar chlorophyll (a, b and total) content of the flag leaf (11.6, 19.7 & 31.30 at 2 DAF ; 11.89 ,19.20 & 31.09 at 9 DAF; 11.80, 20.56 & 32.11 at 16 DAF and 11.60, 16.99 & 28.59 mg g^{-1} at 23 DAF respectively in first experiment and 12.50, 21.15 &33.65 at 2 DAF ; 11.98, 20.44 &32.42 at 9 DAF ; 11.42, 20.45 &31.88 at 16 DAF and 10.17, 15.88 &26.05 mg g^{-1} at 23 DAF respectively in second experiment) as that of crop fertilized with 100% RDF and water spray (11.49, 19.72 &31.21 at 2 DAF; 11.81,19.35 &31.16 at 9 DAF ; 11.74, 20.95 &32.69 at 16 DAF and 11.44, 16.87 &28.31 mg g^{-1} at 23 DAF respectively in first experiment and 12.47,21.05 & 33.52 at 2 DAF; 11.83, 20.96 &32.79 at 9 DAF ; 11.48, 20.53 & 32.01 at 16 DAF and 10.32, 15.98 & 26.31 mg g^{-1} at 23 DAF respectively in second experiment) indicating that 7.5 % K sap was capable enough to compensate the 50 % fertilizer requirement of wheat crop. Genard *et.al*. 1991 reported that the chlorophyll content had changed throughout the growing season of plants and many external sources such as seaweed sap which is a marine macro algae extract found in shallow coastal area and it contains many micro and macro elements as well as glycine betains are responsible for slowing down the degradation of leaf chlorophyll rather than increasing its content so that plant maintain its greenness for longer time and delay the loss of photosynthetic activity. Mondal *et.al*. 2015 reported that GA_3 free K sap in maize increase the chlorophyll index over water spray Seaweed extracts are also known to cause many beneficial effects on plants as they contain growth promoting hormones (IAA and IBA, Cytokinins) trace elements (Fe, Cu, Zn, Co, Mo, Mn and Ni), vitamins and amino acids (Challen and Hemingway, 1965). Value of seaweeds as fertilizers was not only due to nitrogen, phosphorus and potash content, but also because of the presence of trace elements and metabolites. The beneficial effect of different components of seaweed extract application may work synergistically at different concentrations, although the mode of action still remains unknown (Fornes *et al.,* 2002). However, several workers reported that application of seaweed extract increased chlorophyll content (Whapham *et al.*,1993 and Thirumaran *et al.,* 2009), which confirms the findings of the present investigation. Kumari and Sekar (2008) also reported that sea weed extract increased the chlorophyll and carotenoid content in the sodium chloride treated okra seedlings which was higher than the control by increasing the synthesis of chlorophyll proteins, the structural component of chloroplast. Zewail, 2014 also reported that there is increase of photosynthetic pigments and total chlorophyll in mung bean with increasing seaweed and amino acid sprayed levels.

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