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ROLE OF ORGANIC FERTILIZERS ON CHLOROPHYLL CONTENT IN RICE (*ORYZA SATIVA* L.)

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ABSTRACT
In order to study the role of organic fertilizers on chlorophyll content in rice, an experiment was carried out in 2008 and 2009, in randomized block design based on 4 replications. Cow manure, poultry manure, rice straw and husk were used for formulation of organic fertilizers. The treatments of organic fertilizers CM, PM, CMR, PMR, and CPMR were used alone at 4t/ha in five treatments. Half dose of CPMR was used with half dose of RDF (N-50, P-25, K-25 kg/ha) at one level and RDF (N=100, P=50, K=50 kg/ha) alone at one level. The plants without treatments considered as control. Chlorophyll content and Grain yield were significantly increased in all the treatments over control. The maximum grain yield (4776.52 kg/ha) was noted in plants treated with CPMR 2t +½ RDF which had more chlorophyll content. An increase in the grain yield at the abovementioned treatments was may be due to the increase of chlorophyll content which provides the energy necessary for plant growth and reproduction.

KEYWORDS: Chlorophyll content, Grain yield, Rice, Organic fertilizers.

INTRODUCTION

In a study on nutrient absorption by crop plant and its relation to chlorophyll content by Follet *et al.* (1981) clearly indicated that chlorophyll coloration is related to the amount of nutrients absorbed by the plant from the soil. Roy and Singh (2006) also reported similar results in barley with vermi-compost applications. They also observed that application of organic manure resulted in higher leaf chlorophyll content than inorganic fertilizer. Deshpande and Devasenapathy (2010) observed that application of green manure along with FYM and poultry manure significantly increased protein and carbohydrate content in rice.

There are some reports, which indicate that organic fertilizers when supplemented with chemical fertilizers give more positive influence on crop growth and increase organic constituents in test plants. In this respect Jogloy *et al.* (2006) reported that the application of animal manure along with chemical fertilizer gave the highest chlorophyll in (*Helianthus tuberosus* L.). Xu *et al.* (2008) found that the organic manure application with chemical fertilizers increased the yield more than chemical fertilizers alone because it increased nitrogen use efficiency of rice. It is documented that higher chlorophyll content observed in *Helianthus tuberosus* due to organic manures + NPK can be attributed to differences in nitrogen content of the organics manures alone and in combination with NPK and its uptake.

The greater chlorophyll values in leaves treated with organic manure + NPK are of importance because photosynthetic activity and crop yield may increase with increased chlorophyll content of leaves (Ramesh *et al.*, 2002).

MATERIALS AND METHODS

This investigation was carried out at Baykola Research Center, Neka, Mazandaran, Iran during years 2008 and 2009. The experimental farm is geographically situated at 36°, 60'N latitude and 53°, 13'E longitude at an altitude of 4 m above mean sea level.

Formulation of organic fertilizers

Cow manure, poultry manure, rice straw and husk were used for formulation of organic fertilizers. These materials were mixed in required proportions and composted for about 40 days. After completion of composting, these were sieved thorough 2 mm mesh and then analysed for their nutrients. The proportions of raw materials used are given in Table-1.

Treatments

Organic fertilizers CM, PM, CMR, PMR, and CPMR were used alone at 4t/ha in five treatments. Half dose of CPMR was used with half dose of RDF (N-50, P-25, K-25 kg/ha) at one level and RDF (N=100, P=50, K=50 kg/ha) alone at one level. The plants without treatments considered as control. The recommended chemical fertilizer dose for rice is N-100: P-50: K-50. This combination was termed as RDF and used in one treatment. Based on RDF amounts the NPK values were calculated from the organic fertilizers analysis and 4 t/ha dose was decided based on NPK content of CMR,

PMR and CPMR and similar volume (i.e. 4 t/ha) of CM and PM organic fertilizers was used for treatment. Nitrogen in the form of urea was used three times during growth season (1st dose at the time of transplanting, 2nd dose at tillering time and 3rd dose at the time of flowering). Hand weeding was done after 3 weeks of transplanting. The pests and diseases were controlled by application of insecticides and tricycasol was used for rice blast.

Table 1. Raw materials used in formulation of organic fertilizers.

Sr. No.	Formulation (Abbreviated Name)	Ingredients				Total
		Cow Manure (kg)	Poultry Manure (kg)	Rice Straw (kg)	Rice husk (kg)	
1	CM	1900	-	-	100	2000
2	CMR	1500	-	400	100	2000
3	PM	-	1900	-	100	2000
4	PMR	-	1500	400	100	2000
5	CPMR	500	900	500	100	2000

(1) CM: Cow manure+ rice husk, (2) CMR: Cow manure+ rice straw and husk,
(3) PM: poultry manure+ rice husk, (4) PMR: poultry manure+ rice straw and husk,
(5) CPMR: Cow manure+ poultry manure+ rice straw and husk

Table 2. Details of the field experiment.

1.	**Season**	**May-August**
2.	**Crop**	*Oryza sativa* L.
3.	**Variety**	Local Tarom
4.	**Plot size**	5 × 2 m
5.	**Crop duration**	90 -95 days
6.	**Date of sowing**	20. 04. 2008-9
7.	**Date of transplanting**	24. 05. 2008-9
8.	**Date of harvesting**	20-25.08.2008-9
9.	**Design**	RCBD
10.	**Number of replications**	4

Estimation of photosynthetic pigments

Chlorophyll were extracted and estimated by Arnon's (1949) method and Coombs *et al.* (1985). One gram of the fresh flag leaf tissue was cut into small pieces and placed into a specimen bottle containing10 ml of absolute ethanol and stored in the dark for two weeks. 1 ml of the filtered extract was then diluted with 6 ml of absolute ethanol and the absorbance of the chlorophyll solution measured using a spectrophotometer (Shimadzu- 1700) at 645 and 663 nm. The chlorophyll a and chlorophyll b content in milligrams (mg) were estimated using the formula of Arnon (1949).

Grain yield (kg/ha): Samples from 1 M² area from each plot were used for Grain yield.

RESULTS

Chlorophyll a (mg g-^{1})

The results pertaining to effect of organic fertilizers, combination of chemical fertilizer with organic fertilizers and recommended dose of NPK on flag leaf chlorophyll a content in rice is given in table 3. Among the treatments the half dose of CPMR+ RDF, RDF and CPMR organic fertilizer show results at par with each other and better than CM, CMR, PM and PMR in both the years and even in pooled means.

Chlorophyll b (mg g^{-1})

The results pertaining to effect of organic fertilizers, combination of chemical fertilizer with organic fertilizers and recommended dose of NPK on flag leaf chlorophyll b content in rice is given in table 3. Among the treatments the half dose of CPMR+ RDF, RDF and CPMR organic fertilizer show results at par with each other and better than CM, CMR, PM and PMR in both the years and even in pooled means.

Total Chlorophyll (mg g^{-1})

The results pertaining to effect of organic fertilizers, combination of chemical fertilizer with organic fertilizers and recommended dose of NPK on flag leaf total chlorophyll content in rice is given in table 3. Among the treatments the half dose of CPMR+ RDF, RDF and CPMR organic fertilizer show results at par with each other and better than CM, CMR, PM and PMR in both the years and even in pooled means.

Table-3: Effect of different levels organic fertilizer, recommended dose of chemical fertilizer and combination of organic and chemical fertilizers on rice (*O. sativa*) leaf chlorophyll a, chlorophyll b and total chlorophyll contents (mg g^{-1}) and grain yield (kg/ha).

Treatments (Per hectare)	Chlorophyll a (mg g^{-1})		Chlorophyll b (mg g^{-1})		Total Chlorophyll (mg g^{-1})		Grain yield (kg/ha)	
	Pooled	PIOC	Pooled	PIOC	Pooled	PIOC	Pooled	PIOC
Control	1.07	0.00	0.707	0.00	1.777	0.00	3943.90	0.00
RDF (NPK) 100:50:50 kg	1.46	36.45	0.898	27.02	2.358	32.70	4582.32	16.19
CM 4t	1.26	17.76	0.779	10.18	2.039	14.74	4044.92	2.56
CMR 4t	1.30	21.50	0.807	14.14	2.107	18.57	4135.21	4.85
PM 4t	1.36	27.10	0.868	22.77	2.228	25.38	4377.07	10.98
PMR 4t	1.39	29.91	0.884	25.04	2.274	27.97	4594.59	16.50
CPMR 4t	1.45	35.05	0.894	26.45	2.339	31.63	4699.31	19.15
CPMR 2t + ½ RDF	1.50	40.19	0.931	31.68	2.431	36.80	4776.52	21.11
CD (0.05)	0.037	-	0.046	-	0.042	-	96.36	_
CD (0.01)	0.066	-	0.082	-	0.075	-	170.13	_

PIOC: Percent Increase over Control

Grain yield (kg/ha)

The organic fertilizers treatments had significant effect on the grain yield. The results in table 3 clearly indicate that application of organic fertilizers increased the grain yield significantly in all the treatments over control. Among the treatments the half dose of CPMR+ half dose of RDF CPMR, PMR and RDF show results at par with each other and significantly better than CM, CMR and PM alone in both the years and even in pooled means.

DISCUSSION

The fertilizers added to soil provides macronutrients and micronutrients, which are assimilated by plants and utilized for various metabolic activities to synthesize chlorophyll, required for their normal growth and developments. Various micronutrients are needed for catalytic activities of enzymes essential for respiration, photosynthesis, flowering, fruit setting and seed filing as well as fight against abiotic and biotic stresses. In present investigation, it was planned to study the effect of organic fertilizers alone and in combination with chemical fertilizer on leaf and grain biochemical attributes in rice. The organic fertilizers based on cow manure (CM, CMR) and poultry manure (PM, PMR)

supplemented with rice husk and straw and a composite organic fertilizer (CPMR) were given at the dose of 4 t/ha. A half dose of organic fertilizer CPMR and half dose of RDF, RDF alone were also used for fertilization in rice and investigated for chlorophylls and grain yield. The results pertaining to flag leaf biochemical parameters like photosynthetic pigments are given in tables 3.

Photosynthetic pigments content increased in all the plants treated with organic fertilizers and chemical fertilizers as compared to the control. The plants treated with CPMR + RDF showed maximum content as compared to control and other treatments. The present investigation indicated that rice plants respond better with respect to chlorophyll content with combination treatment of CPMR+NPK compared to NPK and organic fertilizers alone.

Photosynthesis is a process, which provides the energy necessary for plant growth and reproduction. Among pigments chlorophylls, the green pigments with magnesium at core of heterocyclic protoporphyrin ring, represents the principal pigment responsible for light absorption and photosynthesis (Nelson and Cox, 2004). Photosynthesis however, is a complex process that is sensitive to macronutrients and micronutrients availability (Marschner, 1995).

Chlorophyll content increased in all treatments compared to the control. It showed maximum content in combination treatment as compared to control and other treatments. The present investigation indicated that rice plants responded better with respect to Chlorophyll content with combination treatment of organic fertilizer CPMR + RDF (NPK) compared to NPK and organic fertilizers alone. Amujoyegbe *et al*. (2007) studied the effects of inorganic fertilizers (NPK), poultry manure alone, and in combination on the chlorophyll component of maize and sorghum; they noted that combination treatment exhibited higher chlorophyll a and b in maize and poultry manure alone enhanced those in sorghum. According to them increase in total chlorophyll pigments due to addition of chemical fertilizers to poultry manure increased both chlorophyll a and b and thereby increased the total chlorophyll.

Researchers like Berova and Karanatsidis (2009) and Fernéndez-Luqueño *et al.* (2010) studied the effect of organic fertilizers on the amount of chlorophyll pigments and rate of photosynthesis. These researchers concluded that the application of organic fertilizers can not only enhances the synthesis and amount of chlorophylls but also increases the rate of photosynthesis. Similarly, potash application enhances the uptake of N and hence there is an improvement in growth characters. Phosphorus helps in early establishment of crop and formation of fibrous and strong root system. The strong root system absorbs more nutrients including micronutrients from the soil and finally contributes towards rapid plant growth (Hari *et al.*, 2006). Micronutrients are also reported to have vital role in photosynthesis. Magnesium is principal element required for chlorophyll synthesis, as it is the part of chlorophyll structure. Iron complexes with proteins to form important enzymes in the plant and is associated with chloroplasts, where it has some roles in the synthesizing chlorophyll, hence iron is necessary for the maintenance and synthesis of chlorophyll and RNA metabolism in the chloroplasts. Calcium and copper must be present for iron to function properly. Boron has a vital role in nitrogen assimilation. Fertilizers with micronutrients can increase chlorophyll because the availability of Mg and uptake of Mg by plant (Suntoro, 2002). Magnesium is involved in numerous physiological and biochemical processes activating more enzymes than any other mineral nutrient, thus, making a significant contribution to plant growth and development (Epstein and Bloom, 2004). Zinc enhances the cation-exchange capacity of the roots, which in turn enhances the absorption of essential nutrients, especially of nitrogen that is responsible for higher protein content. The increase in nitrogen uptake might be due to increase in sulphur content resulting in the stimulation of protein synthesis and due to its synergistic effect on the utilization of nitrogen (Singh *et al.*, 2010**).**

This suggests that nutrients released by combination treatment were available to synthesize more chlorophyll and develop the site of photosynthesis. A promotion effect of organic and inorganic fertilizers on chlorophyll contents might be attributed to the fact that N is a constituent of chlorophyll molecule. Moreover, nitrogen is the main constituent of all amino acids in proteins and lipids that act as a structural compound of the chloroplast (Arisha and Bradisi, 1999).

Regarding to the key role of nutrients, such as nitrogen (protein, enzymes and chlorophyll synthesis), potassium (N uptake), phosphorous (strong root system), magnesium and iron (chlorophyll synthesis), zinc, manganese (cation-exchange and enzyme activities), calcium and copper (for functioning of iron) and boron (N assimilation); these nutrients might have absorbed by plants through fertilizers, which worked in coordination and contributed in chlorophyll structure and synthesis. And this could be the reason for more chlorophyll in plants treated with half dose of CPMR and RDF, where RDF might have supplied NPK during early growth and CPMR might have supplied

micronutrients essential for synthesis and increasing leaf chlorophyll pigments. Increased photosynthetic pigment in 2 t/ha CPMR+½RDF, NPK and CPMR organic fertilizers treatment may be attributed to improved soil physical and chemical properties and optimum nutrients availability in fertilizers.

CONCLUSION

From aforementioned data, we conclude that CPMR supplemented with RDF fertilizer can fulfil the requirement of essential macro and micronutrients at proper growth stages. In present investigation the half doses of CPMR and RDF showed significant enhancement for chlorophyll a, chlorophyll b and total chlorophyll which is followed by RDF and CPMR. Hence more productivity in rice better grain yield was observed in these treatments.

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