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Elucidating the effect of integrated nutrients, farm yard manure and zinc on physiology of rice variety Pant Dhan 4

Neelam Pankaj[1], Reshu Saxena[2]
1-Deptt. of Plant Physiology, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture & Technology, Pantnagar, U.S. Nagar. 263145, Uttarakhand, India
2- Deptt of Microbiology, College of Basic Science and Humanities, G. B. Pant University of Agriculture & Technology, Pantnagar, U.S. Nagar. 263145, Uttarakhand, India
Email: candypankaj123@gmail.com

ABSTRACT
Increased use of chemical fertilizers is seen around the world in last few decades. Although fertilizers are important for enhancing crop production but excessive use causes deterioration in soil quality and poor crop productivity. Application of organic fertilizers has been seen to gradually bring an improvement in soil productivity and crop performance. Leaf colors of a plant can be used to determine stress level due to its adaptation to environmental change. For most of the leaves, green-related colors are sourced from chlorophyll a and b. In present study, chlorophyll content was measured at the maximum tillering stage of rice (Oryza sativa L.). Results showed that the plants with an integrated dose of NPK+Zn+FYM had elevated chlorophyll content. The purpose of this research is to investigate the effect of organic fertilizer and dosage of urea fertilizer on growth, yield, and quality of rice, microbial populations, and soil respiration and explain the changes in activities of the key enzymes responsible for nitrate reduction in rice. The results indicate that rice plants fed by an integrated dose of fertilizer along with Zn and Farm Yard Manure, exhibit a simultaneous increase in nitrate reductase activity. Rice production using an organic fertilizer is expected to reduce the need for urea fertilizer thereby improving soil fertility and health to support growth and increase the production of rice variety pant dhan 4.
KEYWORDS: *Chlorophyll, Rice, Nitrate Reductase, Days to flowering, Days to maturity.*

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INTRODUCTION

Application of fertilizers have been a common practice to use as an alternative source for nutrients in nutrient-deficient soils. Because of higher risk and reduced efficiency, most rain-fed lowland rice farmers apply different fertilizers in a much smaller amount than in irrigated rice system [1].Fertilizer use for rice crop constitute about one-third of total fertilizer available in India. The major nutrients required by rice are nitrogen, phosphorus, and potassium. Among the micronutrients, zinc is most important. Micronutrients in crop production are important and deserve consideration similar to that of macronutrients [2]. Chlorophyll is a green photosynthetic pigment which helps plants to get energy from light and sustain their life process. There may be many factors that affect the photosynthesis; the main factors are light intensity, carbon dioxide concentration, and temperature [3]. The correlation between leaf area and yield suggests chlorophyll and leaf area as important parameters in determining the yield [4].Nitrogen is the most vital nutrient needed for plant and is required for successful plant growth and development. Urea should be given sufficiently without damaging soil fertility [5]. Chemical fertilizer as a chief fertilizer of modern agriculture is the major supplier of nutrients besides organic and green manures which although boosted the crop production but at the same time, it led to the severe negative effects on physio-chemical properties of soil, nitrogen transformation, macro, and micronutrient uptake and nutritional composition [6]. Application of suitable fertilizers is one of the ways to attain maximum crop yield. It was reviewed that integrated use of organic manure and chemical NPK fertilizers would be quite promising not only in providing greater stability in production but also in maintaining a better soil health and fertility. Organic matter has an important role in maintaining soil fertility and productivity [7,8,9]. Intensive cropping with HYV of rice leads to nutrient deficiency and nutrient mining and this

nutrient imbalance can be minimized by wise application of nutrients through organic manures. Losses of soil organic matter can only be replenished in short term by application of organic matter such as manures [10].
The production of rice is, however, facing a sustainability problem due to practices of modern production system with indiscriminate use of chemical fertilizers and pesticides [11]. Hence, the present study was planned with an objective to investigate the effect of organic manures and chemical fertilizer along with Zn on the yield of Rice verity pant dhan 4.

MATERIAL AND METHODS

A study was conducted at the Norman E. Borlaug Crop Research Centre (CRC) and Department of Plant Physiology, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar District (Uttarakhand) INDIA at Kharif season during 2010-11 to investigate the comparative use of nutrient management. The experiment was laid out with fourteen treatments in randomized block design. Treatment combination constitute of T_1(control), $T_2(N_{120})$, $T_3(N_{120}P_{40})$, $T_4(P_{40}K_{40})$, $T_5(N_{120}K_{40})$, $T_6(N_{120}P_{40}K_{40})$, T_7 ($N_{120}P_{40}K_{40}$+Zn), T_8($N_{120}P_{40}K_{40}$+FYMr), T_9 ($N_{120}P_{40}K_{40}$+ZnF+FYMr) T_{10} (N_{180} P_{80} K_{40}+Znf+FYMr), T_{11}(N_{150} $P_{40}K_{40}$), T_{12} (N_{180} P_{80} K_{40}+Znf), T_{13} (N_{180} P_{80} +ZnF), T_{14}{$N_{120}P_{40}K_{40}$ (DAP)}.N= Nitrogen, P=P_2O_5, K=K_2O, Zn= Zinc, f= foliar application, DAP=Diammonium Phosphate. Nitrogen, phosphorous, potassium and zinc were supplied through urea, single super phosphate, muriate of Potash and zinc sulphate (LR grade) respectively. Different nutrient treatments were given to the rice crop variety Pant Dhan 4.

Chlorophyll content:

The concentration of chlorophyll *a* (*Chla*), chlorophyll *b* (*Chlb*) and total chlorophyll were determined according to the method given by Hiscox and Israelstam [12]. 50 mg of leaf samples were placed in a test tube, then 10 ml of DMSO was added and then incubated at 65^0 for 3 hrs in an oven. After 3hrs, The *Chl a* and *Chl b* concentrations were measured using a UV-visible spectrophotometer (Thermospectronic Biomate 5) at wavelengths 663 nm and 645 nm. A solution of pure DMSO was used as a blank. The *Chl a*, *Chl b* and total chlorophyll (mg g^{-1} FW) concentrations in the leaf tissues were calculated according to the following equations:
Chlorophyll ‘a’ (mg/g FW) = $(12.7 \times A_{663} - 2.69 \times A_{645})\ V/1000 \times W$
Chlorophyll ‘b’ (mg/g FW) = $(22.9 \times A_{645} - 4.68 \times A_{663})\ V/1000 \times W$
Total Chlorophyll (mg/g FW) = $(20.2 \times A_{645} + 8.02 \times A_{663})\ V/1000 \times W$
Where,
A_{645} =Absorbance at 645 nm
A_{663} =Absorbance at 663 nm

Nitrate Reductase

Reagents used

1. Phosphate Buffer (0.2 M, pH 7.5) Reagent A: 8.804 g of potassium dihydrogen phosphate (KH_2PO_4) was dissolved in 250 ml of distilled water.
   Reagent B: 8.709g of dipotassium hydrogen phosphate (K_2HPO_4) was dissolved in 250 ml distilled water. The desired buffer was obtained by mixing 16ml of reagent A & 84 ml of reagent B to adjust the pH 7.5
2. Potassium Nitrate (KNO_3) (0.4M): 4.044g KNO_3 dissolved in 100 ml of distilled water.
3. NEDD (N-1Nepthyl ethylene diamminedihydrochloride solution) 0.2%w/v 200 mg NEDD was dissolved in 100ml distilled water and kept in an amber colored bottle.
4. Sulfanilamide Solution (1%w/v) 1g Sulfanilamide was dissolved in 100 ml 1.5 N HCl

Assay of NR enzyme activity,

To estimate the NR activity, 0.5 g of freshly harvested leaf tissue was cut into small pieces and transferred into a beaker containing 3ml each of KNO_3 and phosphate buffer (0-4°C) the reaction mixture was degassed for infiltration of the substrate into the tissue. It was then incubated in dark at 33°C 30 minutes water bath. Aliquot of 0.2ml, twice at 10 min and 40 min after incubation was taken in separate test tubes containing 1ml distilled water. Add 2ml [1:1(V/V)] mixture of NEDD and sulfanilamide and kept in test tubes in dark for pink color development. The absorbance was measured at 540nm with the help of spectrophotometer using distilled water as blank. The amount of nitrate produced as g fresh weight of the sample after 10 & 40 min calculated by following formulae.
μ mol NO_2 /g FWat 10 min = absorbance at 540nm 10 min sample)×0.081×25.8×1/0.2
μ mol NO_2 /g FWat 40 min = absorbance at 540nm (40 min sample)×0.081×25.8×1/0.2

NR enzyme activity = 2(Nitrate produced at 40 min-nitrate produced at 10 min) μ mol NO_2 /g FW per hour.

Days to general flowering.

Days to general flowering were recorded by calculating the number of days from date of sowing to the date heading. It is the stage when 50% of the panicles in the plot are exerted.

Days to maturity.

Stage of maturity was attained when 90% of the panicles turn ripe. Days to maturity were recorded by calculating the number of days from the date of sowing to the date of maturity.

RESULTS

Results are summarized in Table 2 and Table 2 below displaying the effect of nutrients on different physiological parameters in rice variety Pant Dhan 4:

Table 1: Effect of different nutrients on Chlorophyll 'a' mg/g fresh weight, Cholorophyll b mg/g fresh weight, total chlorophyll on rice variety Pant Dhan 4 in *Kharif* 2010 and 2011.

S.No.	Treatments	Chlorophyll 'a' mg/g fresh weight		Cholorophyll b mg/g fresh weight		Chlorophyll total mg/g fresh weight	
		2010	2011	2010	2011	2010	2011
T_1	Control	.024±0.28	0.055±0.14	0.14±0.048	0.16±0.12	1.143±0.24	1.125±0.041
T_2	N	1.256±0.22	0.850±0.28	0.31±0.13	0.38±0.070	1.572±0.25	1.193±0.23
T_3	NP	1.154±0.23	0.931±0.25	0.17±0.063	0.27±0.15	1.324±0.18	1.275±0.18
T_4	PK	0.889±0.057	1.039±0.14	0.32±0.093	0.26±0.10	1.215±0.039	1.336±0.055
T_5	NK	1.295±0.21	0.985±0.16	0.26±0.048	0.39±0.13	1.508±0.17	1.461±0.12
T_6	NPK	1.274±0.22	1.030±0.22	0.17±0.044	0.26±0.13	1.384±0.19	1.336±0.12
T_7	NPK+Zn	1.000±0.27	1.200±0.16	0.57±0.20	0.26±0.10	1.420±0.15	1.344±0.13
T_8	NPK+FYM	1.185±0.26	0.993±0.23	0.35±0.057	0.51±0.11	1.464±0.23	1.506±0.20
T_9	NPK+Znf+FYM	1.156±0.26	1.050±0.30	0.27±0.095	0.35±0.069	1.430±0.20	1.285±0.18
T_{10}	$N_{180}P_{80}$K+Znf+FYM	0.957±0.19	0.901±0.17	0.31±0.64	0.36±0.090	1.976±0.73	1.173±0.052
T_{11}	N_{150}PK	1.066±0.32	1.092±0.15	0.53±0.15	0.34±0.059	1.598±0.23	1.324±0.12
T_{12}	$N_{180}P_{80}$K+Znf	1.613±0.098	0.959±0.20	0.20±0.087	0.34±0.68	1.793±0.91	1.259±0.76
T_{13}	$N_{180}P_{80}$+Znf	0.921±0.25	0.833±0.16	0.47±0.05	0.43±0.03	1.491±0.21	1.264±0.16
T_{14}	NPK (DAP)	0.918±0.092	1.354±0.20	0.30±0.05	0.28±0.08	1.226±0.071	1.615±0.11
	S.Em.±	0.062	0.084	0.029	0.026	0.069	1.125
	CD at 5%	0.177	0.241	0.083	0.075	0.199	1.193

Table 2: Effect of different nutrients on Nitrite Reductase Enzyme Activity µmol NO_2 /g fresh wt./hour, days to general flowering and days to maturity on rice variety Pant Dhan 4 in *Kharif* 2010 and 2011

S.No.	Treatments	Nitrite Reductase Enzyme Activity µmol NO_2 /g fresh wt./hour		Days to general flowering		Days to maturity	
		2010	2011	2010	2011	2010	2011
T_1	Control	2.18±0.32	2.953±0.86	94±1.04	100.00±4.26	121.00±1.82	126.00±0.91
T_2	N	3.15±0.47	4.328±1.09	94±1.04	101.50±2.90	126.25±1.49	127.75±0.75
T_3	NP	3.98±0.48	4.350±0.61	99±1.25	99.75±3.25	119.00±1.87	125.00±1.22
T_4	PK	3.22±0.36	2.631±0.55	98±0.85	99.25±2.59	116.75±1.70	126.50±1.65
T_5	NK	3.01±0.15	2.981±0.21	107±1.04	102.50±1.32	129.75±1.03	126.25±2.83
T_6	NPK	3.77±0.20	4.376±0.76	92±1.37	99.25±2.25	124.25±2.65	128.50±1.19
T_7	NPK+Zn	2.76±0.80	2.752±0.56	104±1.55	100.50±1.50	126.25±1.49	127.25±1.10
T_8	NPK+FYM	2.75±0.62	3.791±0.30	94.1±1.19	101.50±1.55	124.25±2.17	127.50±2.02
T_9	NPK+Znf+FYM	2.79±0.32	3.031±0.43	108±2.65	101.50±3.22	122.00±1.47	128.50±1.55
T_{10}	N_{180} P_{80} K+Znf+FYM	3.23±0.22	3.814±0.32	102±1.68	101.00±3.89	128.75±1.70	128.50±1.84
T_{11}	N_{150}PK	3.23±0.48	3.485±0.66	99±2.83	101.00±3.08	130.25±2.13	128.75±1.70
T_{12}	$N_{180}P_{80}$K+Znf	3.42±0.60	3.594±0.89	105±3.68	93.50±3.17	126.25±1.10	126.25±1.43
T_{13}	$N_{180}P_{80}$+Znf	3.15±0.090	3.502±0.48	99±2.27	105.00±2.67	127.25±1.93	127.25±1.60
T_{14}	NPK (DAP)	4.40±0.10	3.884±0.37	104±2.25	96.75±1.54	121.50±1.32	127.75±1.49
	S.Em.±	0.243	0.265	2.43	2.42	2.20	1.59
	CD at 5%	0.697	0.760	6.97	6.93	6.32	4.55

DISCUSSION

Effects fertilizers on Chlorophyll content: Chlorophyll pigments play a vital role in the photosynthetic process and biomass production. Genotypes maintaining higher leaf chlorophyll-a, chlorophyll-b, and chlorophyll-total during growth period may be considered a potential donor for the ability to produce higher biomass and photosynthetic capacity.

Chlorophyll a

Data presented in Table 1 which showed that during 2010 and 2011maximum value of chlorophyll 'a' was recorded by N_{180} P_{80} K+Znf (1.613 mg/g fresh wt.) and NPK (DAP) (1.35 mg/g fresh wt.) respectively however least value of chlorophyll 'a' was recorded for control (0.024mg/g fresh wt.) and (0.055 mg/g fresh wt.) for year 2010 and 2011 respectively.

Table shows that if the dose of nutrient is increasing the chlorophyll 'a' also increase, and the maximum chlorophyll 'a' was recorded for the treatment N_{180} P_{80} K+Znf and NPK (DAP)which have all the nutrient comparatively higher amount the increment in chlorophyll 'a' increase in range 19.64% to 40.53%for 2010 and 2011 respectively in comparison to control.

Chlorophyll b

The data presented in Table 1, showed during 2010 and 2011 maximum value for chlorophyll 'b' was recorded by NPK+Zn (0.571 mg/g fresh wt.) and NPK+FYM (0.519 mg/g fresh wt.) respectively however least value of chlorophyll b was recorded by the control (0.14 mg/g fresh wt.) and (0.16 mg/g fresh wt.) for the year 2010 and 2011 respectively. Results showed that if the nutrient dose is increasing chlorophyll 'b' also increasing the increment was in range37.56% to 48.51% for 2011 and 2010 respectively and decrease 72.94% to 40.30% for2010 and 2011 respectively in comparison to control.

Total Chlorophyll

The data presented in Table 1., showed that, maximum value chlorophyll total during 2010 and 2011 for N_{180} P_{80} K+Znf+FYM (1.97 mg/g fresh wt.) and NPK (DAP) (1.615 mg/g fresh wt) respectively while the least value of total chlorophyll showed by control(1.143 mg/g fresh wt.) and (1.12 mg/g fresh wt) for 2010 and 2011respectively. The results reveal that if the dose of nitrogen is increased the rate of total chlorophyll increased in a range of 14.68% to 30.32% for 2010 and 2011 respectively and decreased 35.26%to 1% for 2010 and 2011 respectively.

It is observed that if the nutrient dose was increased chlorophyll total was also increased. Similar results were found which showed that if the following treatment T_1 Control N_0P_0, T_2 N_{40}+P_{40}, T_3N_{80}+P_{40}, T_4N_{80}+N_{80} is given, then the value of chlorophyll total is 2.6, 2.8, 3.1, 3.4 mg/g fresh wt respectively which is getting increased in ascending order [13]. Since nitrogen has a role in the synthesis of chlorophyll where Rubisco enzyme acts as a catalyst in CO_3 fixation that plants need for photosynthesis, hence total nitrogen content in plants can significantly influence the process of photosynthesis via the alterations in photosynthetic enzymes and regulating chlorophyll formation. In plants, nitrogen is initially present in the form of ammonia and subsequently ammonia changes into glutamic acid, catalyzed by the enzyme glutamine. Glutamic acid serves as the base material in the biosynthesis of amino acids and nucleic acids and glutamic acid as a precursor for the porphyrin ring in the formation of chlorophyll. Similar results were found which reveals the fact that for four treatments T_1 Control N_0P_0, T_2 N_{40}+P_{40}, T_3N_{80}+P_{40}, T_4N_{80}+N_{80} chlorophyll an increase in 1.8, 2.00, 2.2 and 2.4mg/g fresh weight respectively which is showing increment in increasing order of dose [13]. Similar results were found which showed that if the following treatment T_1 Control N_0P_0, T_2 N_{40}+P_{40}, T_3N_{80}+P_{40}, T_4N_{80}+$N_{80}$is given, then the value of chlorophyll'b' is 0.761, 0.837, 0.955 and 1.01mg/g fresh wt. respectively [14].

Nitrate Reductase

Two key enzymes are responsible for nitrate assimilation: nitrate and nitrite reductase. Both the expression of NR genes and the rate of nitrate reduction in the plant tissues can be considerably modified by some factors such as light, presence of NO_3- ions, day length, sugar level or certain nitrogen metabolites. Moreover, the form of nutritive nitrogen N-NO_3, N-NH_4, N-NH_2 also affects the activity of NR and NiR. Data presented in Table 2, which showed that during 2010 and 2011 maximum Nitrate Reductase activity was observed for the NPK (DAP) (4.40 μ mol NO_2 /g FW/hour.) and (4.37 μ mol NO_2 /g FW/hour)respectively. However, least value of Nitrate Reductase was recorded by the treatment control (2.18 μ mol NO_2 /g FW/ hour) and PK (2.63 μ mol NO_2 /g FW per hour) for the year 2010 and 2011.The rate of nitrate reduction is an important factor which modifies the content of this compound in plant tissue. Data showed that increment in nitrate Reductase activity was ranged in 32.35% to 9.68% for 2010 and 2011 respectively and decreased from 50.21% to 38.79% for 2011 and 2010 respectively.It was observed that if the amount of nutrient is increasing, the rate of Nitrate Reductase activity also increase and similar result was found in another study where the application of foliar nutrition with urea caused a significant increase in Nitrate Reductase NR activity in broccoli heads in comparison with untreated plants [15].

Days to general flowering

The data presented in 2., showed that during 2010 and 2011 maximum number of days to general flowering was observed for the NPK+Znf+FYM (108) and N_{180} P_{80} +Znf (105) respectively while minimum number of days to general flowering was recorded by the control (94)and N_{180} P_{80} K+Znf (93.50) for 2010 and 2011 respectively. The number of days to general flowering was increased in a range of 12.90% to 4.76%for 2010 and 2011respectively and decreased up to 6.95% for both the year in comparison to control.The number of days to general flowering of rice plant was greatly influenced by different methods of application of nitrogen fertilizer. Results show that the treatment with FYM displayed the maximum number of days to general flowering it is might be because of the slow release of nutrients for a long period. Similar results were found which showed that the days of general flowering of rice was affected by different levels of organic fertilizer and treated manure under aerobic rice technology. It showed that 2 tons treated organic fertilizer/ha was the earliest to flower with 85 days whereas 4 tons of organic fertilizer/ha with 87 days [16].

Days to maturity

The data presented in Table 2. showed that during 2010 and 2011 the maximum number of days to maturity was observed for both the year, by N_{150} PK (130.25) and (128.75) respectively. However, a minimum number of days to maturity was recorded by the PK (116.75) and NP (125.00) for 2010 and 2011. The data presented for days to maturity increased in a range of 7.10% to 2.13% for 2010 and 2011respectively and decreased by 3.6% to 0.8%for 2010 and 2011 respectively in comparison to control.

CONCLUSION

In present study, integrated use of chemical fertilizer with Organic manure display maximum benefits in context of overall crop health and physiological properties. An increase in Chlorophyll content, nitrate reductase activity potentially improves the crop performance with least effect on soil quality. Days to flowering and maturity are affected by slow release of nutrients in soil but overall keep the soil health intact for better crop production. An indepth understanding of microbial populations involved will further contribute to best use of suitable fertilizers and provide better solutions.

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