

Effect of long-term fertilization and manuring on soil enzyme activity in rice-rice cropping system in semi-arid southern India

ABSTRACT

Nutrient management practices have huge influence on soil biological health. The present study was conducted during *kharif*-2021 in the on-going AICRP on LTFE after 21 years of continuous application of fertilizers and manures to estimate the enzymatic activities i.e., urease and dehydrogenase activity at different growth stages of rice crop. In all the treatments, enzymes exhibited higher activity at flowering stage and thereafter the activity was decreased towards harvesting stage of the rice crop. Among all the treatments, FYM and 100% NPK+FYM treatments recorded highest dehydrogenase and urease activity, respectively as compared with all other treatments. Balanced fertilizer application i.e., 100 % NPK also recorded significantly higher enzymatic activity compared with imbalanced fertilizer treatments i.e., 100% N and 100% NP during all the stages of crop growth period. Integrated nutrient management (100%NPK+FYM), FYM and 100 % NPK are the best treatment with respect, to increasing enzymatic activity as compared with all other treatments. Grain yield of rice varied from 4078 kg ha⁻¹ to 6569 kg ha⁻¹ during *kharif*-2021. Among all the treatments, maximum grain yield (6569 kg ha⁻¹) was obtained with 100% NPK+FYM. Lower grain yield (4078 kg ha⁻¹) was recorded in the treatment 100% N. Integrated use of inorganic fertilizer and organic manure resulted in higher growth parameters and maximum yield of rice. The combined use of organic as well as inorganic source of plant nutrients could be a sustainable option for optimizing the yield of rice in rice-rice cropping system in semi-arid southern India.

Key words: LTFE, urease, dehydrogenase, FYM and NPK

INTRODUCTION

Rice-rice system is an important cropping system in India. A rice crop removes about 22kg N, 7kg P₂O₅, 32kg K₂O, 1kg S and 40g Zn from soil to produce one tonne of rice (Chaudhary *et al.*, 2007) [1]. Continuous mono cropping of rice with high yielding improved varieties and application of NPK fertilizers has resulted in stagnated or declined productivity (Ladha *et al.*, 2003) [2] which has been ascribed to declined soil fertility with reduced nutrient supplying capacity. Long-term fertiliser experiments conducted throughout the world have revealed that continuous application of NPK alone has caused many damages to physical and biological health (Dey *et al.*, 2015) [3] of soil with reduced supply of N and depletion of total soil K and deficiency of nutrients like sulphur and micronutrients due to their continuous removal from soil (Balu Ram *et al.*, 2014) [4]. For sustainability of such a production system, improvement of soil environment is very important and therefore long-

term experiments with more balanced nutrition including maintaining the soil health are required for managing such a fragile eco system.

Soil enzymes play the key biochemical functions in the overall process of organic matter decomposition in soil system (Mandal *et al.*, 2013) [5]. These enzymes are constantly being synthesized, accumulated, inactivated and/or decomposed in the soil, hence playing very important role in crop production particularly in nutrients cycling (Tabatabai, 1994) [6]. The enzyme dehydrogenase is considered to exist as an integral part of intact cells but does not accumulate extracellular in the soil. Therefore, it is commonly used as an indicator of biological activity in soils (Burns, 1978) [7] urease enzyme is responsible for the hydrolysis of urea fertiliser applied to the soil into NH_3 and CO_2 with the concomitant rise in soil pH (Andrews, 1989) [8]. With this background present investigation was planned with objective to study the influence of different nutrient management practices on soil enzymatic activities at various growth stages of rice.

MATERIALS AND METHODS

The study was undertaken in an ongoing Indian Council Agricultural Research (ICAR) funded All India Coordinated Research Project (AICRP) on Long Term Fertilizer Experiment (LTFE) during *kharif*-2021 at Regional Agricultural Research Station, Jagtial (India) on 21-year-old experiment which was initiated in 2000-2001. The soil was Typic Ustochrept and clay in texture. The experimental site consisted of 12 treatments which were arranged in a randomised block design with three replications. The dimensions of the experimental plot are 12m x 9m (108m²). The treatment details are 50%NPK, 100%NPK (120N:60P₂O₅:40K₂O kg ha⁻¹), 150%NPK, 100%NPK+HW, 100%NPK+Zn, 100%NP, 100%N, 100%NPK+FYM, 100%NPK-S, FYM, Control, Fallow. The soil samples were collected and an assay for soil enzymatic activities *viz.* urease, dehydrogenase, at initial sample (*kharif* - 2021), maximum tillering stage, flowering stage and at harvest.

COLLECTION OF SOIL SAMPLES

Soil samples (wet samples) were collected from the surface soil of experimental area from the different treatments at different growth stages of the rice crop *viz.* initial sample (*kharif* - 2021), maximum tillering stage, flowering stage and at harvest. The samples were stored in the refrigerator at 4°C. Enzymatic activity *viz.* urease and dehydrogenase activity were determined by procedures as follows

Soil enzymes *viz.* urease activity was assayed by the quantifying the amount of ammonium released and expressed as $\mu\text{g NH}_4^+$ released g^{-1} soil h^{-1} described by Tabatabai and Bermner (1972) [9].

Dehydrogenase assays based on the 2,3,5-triphenyl tetrazolium chloride (TTC) to the creaming red coloured triphenyl formazone (TPF) and it was quantifying the amount TPF released and expressed as of $\mu\text{g TPF}$ produced g^{-1} soil day^{-1} described by Cassida *et al.*, (1964) [10].

RESULTS AND DISCUSSION

Dehydrogenase

Dehydrogenase enzyme is known to oxidize soil organic matter by transferring protons and electrons from substrates to acceptors. These processes are part of respiration pathways of soil micro-organisms and are closely related to the type of soil and soil organisms, studies on the activities of dehydrogenase enzyme in the soil is very important as it may give indications of the potential of the soil to support biochemical processes which are essential for maintaining soil fertility.

In the present study highest dehydrogenase activity was observed at flowering stage in all the treatment when it is compared with different crop growth stages rice crop *i.e* initial, maximum tillering stage, flowering stage and at harvest. Highest dehydrogenase activity was observed in the FYM treatment fallowed by 100% NPK+FYM. Dehydrogenase activity decreased after flowering stage to at harvesting stage (Table.1 and Fig. 1). The significant increase of dehydrogenase activity in the treatments FYM and NPK+FYM might be due to addition of organic matter through FYM which increased the microbial activity and microbial biomass (Anantha *et al.*, (2018) [11] and Anantha *et al.*, 2022) [12]). Similar results were also reported by Sheng *et al.*, (2005) [13] and Bhavani *et al.*, (2017) [14]. The higher dehydrogenase activity of 2.89, 6.23, 10.29 and 3.75 $\mu\text{g TPF}$ produced g^{-1} soil day^{-1} were recorded in FYM treatment. The lowest dehydrogenase activity of 1.41, 2.14, 5.11, 1.89 $\mu\text{g TPF}$ produced g^{-1} soil day^{-1} were recorded at initial, maximum tillering stage, flowering stage and at harvest respectively in control plot (Table.1 and Fig.1). Significant effect was observed in between control and 100% N treatments. The treatment 100% N recorded significantly lower dehydrogenase activity than other imbalanced fertilizer treatment of 100% NP. The lower activity of dehydrogenase under 100% N showed the direct inhibitory effect of imbalanced fertilization in making the carbon less available, increase in the retention of

carbon and partial sterilization affect from the increased osmotic potential of soil solution due to the fertilizer salts. These conditions decreased the activity of dehydrogenase in the soil. The dehydrogenase activity was increased with increasing graded levels of NPK i.e., from control to 100 % NPK and the dehydrogenase activity was increased from 1.41 $\mu\text{g TPF produced g}^{-1} \text{ soil day}^{-1}$ (control), 6.99 $\mu\text{g TPF produced g}^{-1} \text{ soil day}^{-1}$ (50% NPK) and 9.84 $\mu\text{g TPF produced g}^{-1}$ (100% NPK) at flowering stage of the rice crop. This confirms that application of balanced fertilizers NPK either alone or in combination with organic manures maintained active pools of C and N in the soil surface layer due to increased plant biomass addition in these treatments. This indicated that organic pools of carbon associated nutrients, particularly nitrogen, could be maintained in rhizosphere zone for maintaining soil organic matter and enzyme activity there by sustaining soil quality in these treatments. Similar findings were also reported by Majhi *et al.* (2019) [15] found that dehydrogenase activity was highest in soil that received organic manure FYM @10t ha⁻¹ yr⁻¹. The increased dehydrogenase activity in organic manure applied soil might be due to incorporation of organics and owing to increase in microbial activity of soil.

UREASE ACTIVITY

In all the fertilizer and manure treatments, urease enzyme exhibited higher activity at flowering stage and thereafter the activity decreased towards harvest (Table.2 and Fig.2). Significantly higher urease activity of 2.58, 3.9, 7.11 and 3.76 $\mu\text{g NH}_4^+$ released g⁻¹ soil h⁻¹ was recorded at initial, maximum tillering, flowering and at harvest stages, respectively in the FYM treatment. The lowest urease activity was recorded as 1.6, 1.99, 4.78 and 1.76 ($\mu\text{g NH}_4^+$ released g⁻¹ soil h⁻¹) at initial, maximum tillering, flowering and at harvest stages respectively in control plot (Table.2 and Fig.2). The 100% N and control plots recorded lower urease activity and this could be attributed to lack of sufficient substrate i.e., organic carbon which acts as a source of energy for proliferating microorganisms. Similar results were also reported by Ramalakshmi (2011) [16], Bhavani *et al.*, (2017) [14] and Majhi *et al.*, (2019) [15]. Significant increase of enzyme activity in the treatments 100% NPK+FYM and FYM may be ascribed to the fact that the organic matter added to soil enhances microbial fermentation of the organic compounds producing compounds which are subjected to reduction and oxidation. A number of fermentation products like ethanol, acetate, lactate act as rich energy sources for proliferating microorganisms and the micro-organisms might release these enzymes into the soil for the reactions necessary to release energy. The results of the present

study indicate that profile of activities of urease showed highest activity at flowering stage and after that a decreasing trend was observed.

GRAIN AND STRAW YIELD (kg ha⁻¹)

Grain yield of rice varied from 4078 kg ha⁻¹ to 6569 kg ha⁻¹ during *kharif*-2021 amongst different nutrient concentration alone and along with organics (Fig.3). Among the treatments maximum grain (6569 kg ha⁻¹) were obtained with 100% NPK+FYM. Highest (7057 kg ha⁻¹) and lowest (4661 kg ha⁻¹) straw yield obtained with the treatments 150% NPK and 100%N, respectively. Grain and straw yield of rice increased significantly with increasing level of fertilizers. This may be due to the higher available nutrients and optimum soil properties in the plots receiving optimum dose (100% NPK) of inorganic fertilizers. Similar results were also reported by Kiranreddy *et al.*, (2018) [17] and Venureddy *et al.*, (2017) [18]. Integrated use of inorganic fertilizer and organic manure resulted in higher growth parameters and maximum yield of rice. Thus, the combined use of organic as well as inorganic source of plant nutrients could be a sustainable option for optimizing the yield of rice.

CONCLUSION

From the foregoing discussion, it is clear that in all the different manure and fertilizer treatments were exhibited more soil enzymatic activity (Urease and Dehydrogenase) at flowering stage and there after activity was reduced towards harvest. Integrated application of fertilizers along with organic manures (100% NPK + FYM) registered significantly highest enzymatic activity and grain yield followed by recommended dose of fertilizer (100% NPK). The combined application of organic and inorganic sources of plant nutrients could be a sustainable option for optimizing soil enzymatic activity and yield of rice.

REFERENCES:

1. Chaudhary SK, Thakur SK, Pandey AK. Response of wetland rice to nitrogen and zinc. *Oryza*. 2007; 44(1): 31-34.
2. Ladha JK, Dawe D, Pathak H, Padre, AT, Yadav RL, Singh B, Singh Y, Singh, P, Kundu, AL, Sakal R, Regmi AP, Gami SK, Bhandari AL, Amin R, Yadav, CR, Bhattarai EM, Das S, Aggarwal HP, Gupta RK, Hobbs PR. How extensive are yield declines in long-term rice-wheat experiments in Asia? *Field Crops Research*, 2003; 81: 159–180.
3. Dey A, Dasgupta S, Bhattacharya S, Krishna Chaitanya A, Pati S, Pal B. Soil physical fertility and performance of potato crop as affected by integration of organic and inorganic fertilizers in new alluvial soils of West Bengal. *Journal Crop and Weed*. 2015;11(1): 132-137.

4. Balu Ram, Singh SK, Latore AM, Kumar O. Effect of Sulphur, Zinc and Boron Application on Growth and Yield of Hybrid Rice. *Journal of Indian Society of Soil Science*. 2014 ;62 (2): 184-188.
5. Mandal KG, Majhi P, Sahoo DK, Rout R, Kumar A, Shosh S, Mohanty RK, Raychaudhuri M. Assessing the soil environment under major cropping systems in Kuanria canal command. *Eco. Env. & Cons*. 2013;19(2): 509-513.
6. Tabatabai MA. Soil enzymes. In: Weaver, R.W., Angle, J.S., Bottomley PS (eds) *Methods of Soil Analysis, Part 2. Microbiological and biochemical properties*. SSSA Book Series No. 5. Soil Sci. Soc. Am. Madison, Wis.1994; 775-833.
7. Burns RG. *Soil Enzymes*. Academic Press, New York. 1978; 370.
8. Andrews RK, Blakeley RL, Zerner B. Urease: A Ni (II) metalloenzyme. In *The Bioinorganic Chemistry of Nickel*, ed. J. R. Lancaster. 1989;141-166.
9. Tabatabai MA, Bremner JM. Michaelis constants of soil enzymes. *Soil Biol. Biochem*. 1972;3: 317-323.
10. Cassida LE, Klein DA, Santoro J. Soil dehydrogenase activity. *Soil Science*.1964;98:371-376.
11. Krishna CA, Majumder SP, Padhan D, Badole S, Datta A, Mandal B, Kiran Reddy, G. Carbon dynamics, potential and cost of carbon sequestration in double rice cropping system in semi-arid southern India. *Journal of Soil Science and Plant Nutrition*.2018;18 (2): 418-434.
12. Anantha KC, Mandal B, Badole S, Majumder SP, Datta A, Padhan D, Babu MVS. Distribution of sequestered carbon in different pools in Alfisols under long-term groundnut system of hot arid region of India. *European Journal of Agronomy*. 2022;135: 126467.
13. Sheng ZL, Min L, Cheng Li C, Yong HC. Variation of soil microbial biomass and enzyme activities at different growth stages of rice (*Oryza sativa*). *Rice Science*.2005;12(4):283-288.
14. Bhavani S, Shaker KC, Jayasree G, Padmaja B. Effect of long-term application of inorganic and organic fertilizers on soil biological properties of rice. *Journal of Pharmacognosy and Phytochemistry*.2017; 6 (5):1107-1110
15. Majhi P, Rout K, Nanda G, Singh M. (2019). Soil Quality for Rice Productivity and Yield Sustainability under Long-term Fertilizer and Manure Application. *Communications in Soil Science and Plant Analysis*. 2019;1–14.
16. Ramalakshmi Ch. S. Vermicomposting for effective waste management and its evaluation under INM rice-pulse cropping system. M.Sc. (Ag.) Thesis. 2011. Acharya N G Ranga Agricultural University, Rajendranagar, Hyderabad, India.
17. KiranReddy G, Uma Rajashaker A, Muneshwarsingh. Effect of long-term fertilizer use on yield and nutrient uptake in rice-rice cropping system. *Progressive research*.2016. 11(Spl. IV): 2661-2664.

18. Venureddy CH, Alok Tiwari, Tedia K, Anil verma, Saxena RR. Long-term fertilizer experiment on soil properties and yield parameters of rice on Chromustert. International Journal of Farm Sciences.2017;7(4):87-91.

Table.1. Effect of continuous application of fertilizer and manures on dehydrogenase activity ($\mu\text{g TPF produced g}^{-1} \text{ soil day}^{-1}$) of various growth stages of rice in a long-term rice-rice cropping system during *kharif*-2021.

Treatments	Initial sample (kharif 2021)	Maximum tillering	Flowering	At harvest
50% NPK	1.74	3.19	6.99	2.31
100% NPK	1.98	4.24	9.84	2.74
150% NPK	1.55	2.15	7.31	1.89
100% NPK+Hw	1.88	3.72	9.27	2.76
100% NPK+Zn	1.94	4.12	9.38	2.99
100% NP	1.86	3.24	8.85	2.24
100% N	1.79	3.18	4.18	1.65
100% NPK+FYM	2.14	5.9	9.89	3.24
100% NPK-S	1.92	3.94	9.35	2.89

FYM	2.89	6.23	10.29	3.75
Control	1.41	2.14	5.11	1.89
Fallow	1.11	1.65	2.18	1.44
SEm(+/-)	0.09	0.08	0.16	0.08
CD@0.05	0.25	0.23	0.46	0.24

Table.2. Effect of continuous application of fertilizer and manures on urease activity ($\mu\text{g NH}_4^+$ released g^{-1} soil h^{-1}) of various growth stages of rice in long-term rice-rice cropping system.

TREATMENTS	Initial	Maximum tillering	Flowering	At harvest
50% NPK	2.00	3.38	6.25	2.51
100% NPK	2.11	3.75	6.99	2.71
150% NPK	2.18	4.02	7.24	2.97
100% NPK+Hw	2.3	3.77	7.01	2.80
100% NPK+Zn	2.26	3.58	7.03	2.86
100% NP	2.06	3.11	6.62	2.69
100% N	2.29	2.99	5.78	2.42
100% NPK+FYM	2.37	4.15	8.15	3.96
100% NPK-S	2.05	3.44	7.13	2.62

FYM	2.58	3.9	7.11	3.76
Control	1.6	1.99	4.78	1.76
Fallow	1.75	1.89	1.92	1.81
S.Em. +/-	0.08	0.15	0.12	0.33
CD@ 5%	0.25	0.45	0.36	0.96

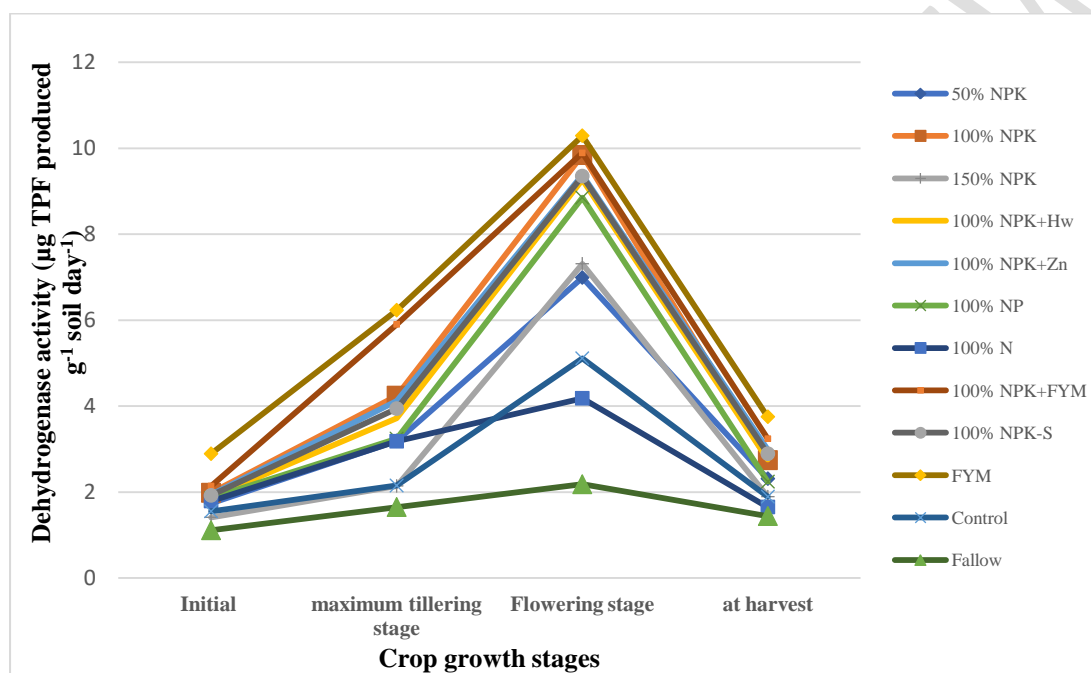


Fig.1.Effect of continuous application of fertilizer and manures on dehydrogenase activity ($\mu\text{g TPF produced g}^{-1} \text{ soil day}^{-1}$) of various growth stages of rice in long-term rice-rice cropping system.

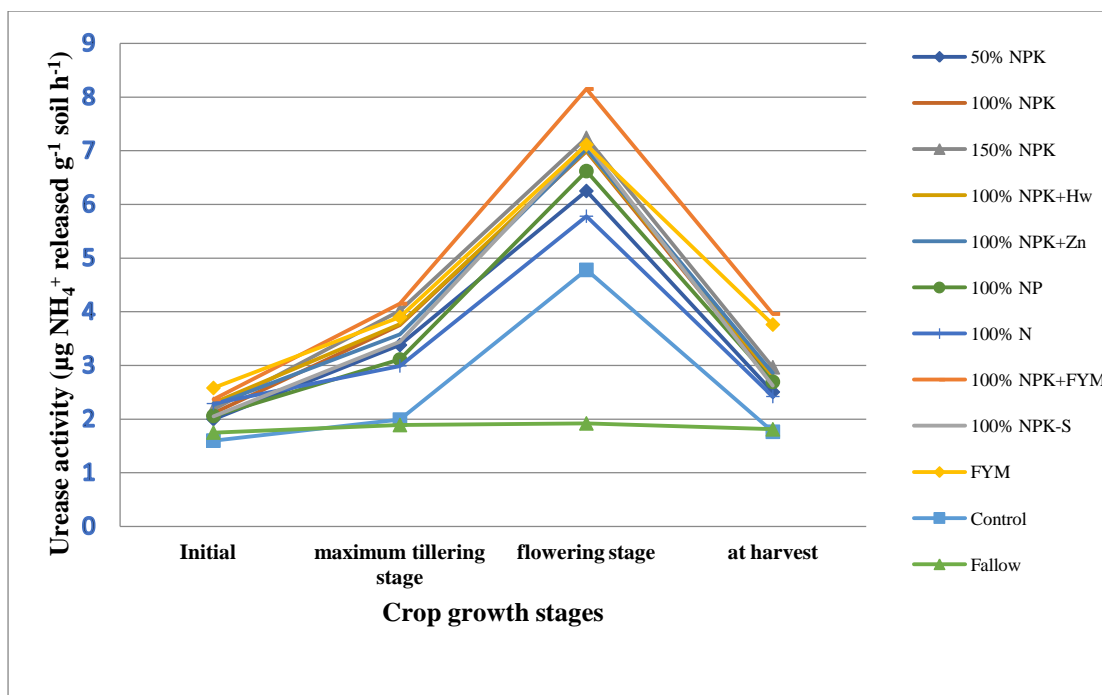


Fig.2 Effect of continuous application of fertilizer and manures on Urease activity ($\mu\text{g NH}_4^+$ released g^{-1} soil h^{-1}) of various growth stages of rice in long-term rice-rice cropping system.

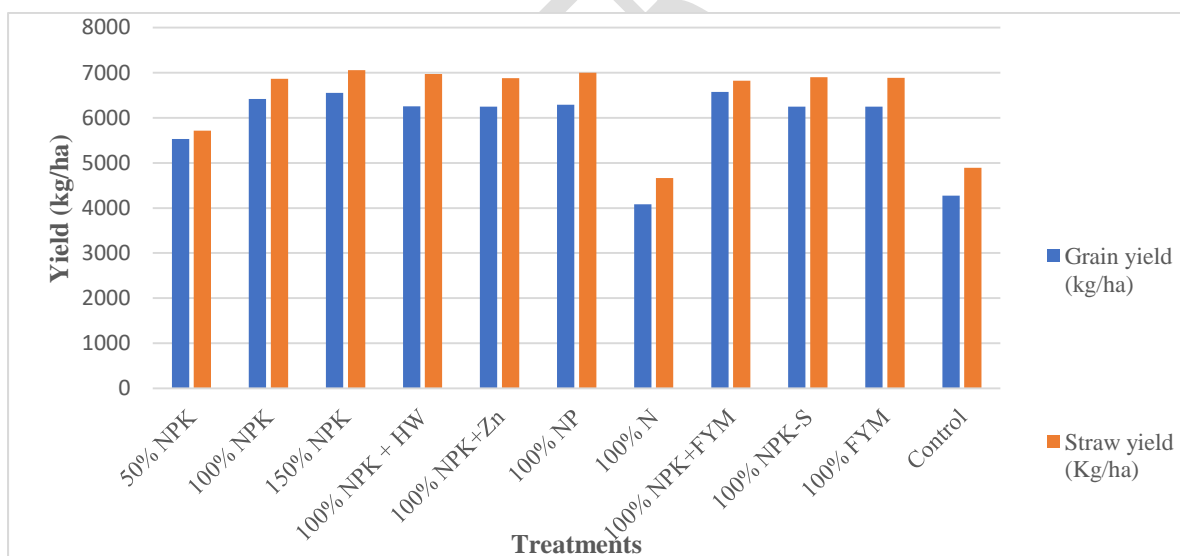


Fig.3. Effect of long-term fertilizers on grain yield (kg ha^{-1}) and straw yield (kg ha^{-1}) in rice-rice cropping system.