Organic Fertilizers Role on Antioxidant Enzymes in Rice (Oryza sativa L.)

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ABSTRACT: In order to study organic fertilizers role on antioxidant enzymes in rice, an experiment was carried out in 2010 and 2011, 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. CPMR 2t was used with ½ 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. Antioxidant enzymes 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. An increase in the grain yield at the abovementioned treatments was may be due to the increase of antioxidant enzymes which protected plant from the stresses.

Keywords: Antioxidant enzymes, Organic fertilizers, Rice, Grain yield

INTRODUCTION

Enzymes are vitally important to the existence of life itself. Enzymes are part of the multi-component defense system and are involved in defense reactions of plants against pathogens and every kind of stress factors. Peroxidases are involved in several physiological and biochemical processes such as cell growth and expansion (Lin and Kao, 1999), differentiation and development, auxin catabolism (Mansouri et al., 1999), lignifications (Sitbon et al., 1999), as well as abiotic and biotic stress responses (Medina et al., 1999). Peroxidases can form a new cell wall through biosynthesis. In the large family of peroxidases, the process of detoxification of \text{H}_2\text{O}_2 is particularly important (Ranieri et al., 2000). In the cell wall, POXs are present in soluble forms. The cell wall stiffening has been attributed mainly to peroxidases whose activity can be detected using the enzymatic assay mixture (Herbette et al., 2003). Peroxidases (POXs) are the most important part of the multiple plant defense system and are mostly synthesized in the chloroplasts (Gabara et al., 2003). Catalase (CAT) is an antioxidant enzyme, which effectively remove the excess \text{H}_2\text{O}_2, and give protection to membranes. Superoxide dismutase enzymes (SODs) act as antioxidants and protect cellular components from being oxidized by reactive oxygen species. SOD enzyme family maintains normal physiological conditions and deal with stress.

(Ahmed et al., 2010) reported that application of organic manures and biofertilizers increase Catalase and Peroxidase activities in sorghum. This result was also supported by (Abd El-Ghany, 2007). According to them these responses may be attributed as an attempt of the plant to overcome the adverse conditions of some elements required for growth and development of sorghum plants. The increments in these enzymes helped the plant to destroy \text{H}_2\text{O}_2 available in normal or abnormal conditions and maintained the ascorbate pool which in turn led to elevate the plant tolerance and maintained best growth.
According to (Trinchera et al., 2008) chemical fertilization is able to supply greater amounts of nitrogen to plants in a brief period to improve better metabolism without needing for additional activity of the enzymes, however, in case of plants receiving the organic fertilizers, may face a condition of slowly releasing the available nitrogen. This slow release of N may be considered as adverse environmental conditions due to nutrient deficiency where the role of the antioxidant enzymes support plants to become more tolerant against the proposed disturbances in different plant physiological process. Hence in present investigation it was planned to analyze organic constituents and assay antioxidant enzymes in rice under different types of organic fertilizers, without or with RDF and compare these with RDF.

MATERIALS AND METHODS

This investigation was carried out at Baykola Research Center, Neka, Mazandaran, Iran during years 2010 and 2011. 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.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation (Abbreviated Name)</th>
<th>Ingredients</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Cow Manure (kg)</td>
<td>Poultry Manure (kg)</td>
</tr>
<tr>
<td>1</td>
<td>CM</td>
<td>1900</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>CMR</td>
<td>1500</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>PM</td>
<td>-</td>
<td>1900</td>
</tr>
<tr>
<td>4</td>
<td>PMR</td>
<td>-</td>
<td>1500</td>
</tr>
<tr>
<td>5</td>
<td>CPMR</td>
<td>500</td>
<td>900</td>
</tr>
</tbody>
</table>

(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

**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 (1<sup>st</sup> dose at the time of transplanting, 2<sup>nd</sup> dose at tillering time and 3<sup>rd</sup> 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>
<thead>
<tr>
<th>Table 2. Details of the field experiment.</th>
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</thead>
<tbody>
<tr>
<td>1. Season</td>
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<tr>
<td>2. Crop</td>
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<tr>
<td>3. Variety</td>
</tr>
<tr>
<td>4. Plot size</td>
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<tr>
<td>5. Crop duration</td>
</tr>
<tr>
<td>6. Date of sowing</td>
</tr>
<tr>
<td>7. Date of transplanting</td>
</tr>
<tr>
<td>8. Date of harvesting</td>
</tr>
<tr>
<td>9. Design</td>
</tr>
<tr>
<td>10. Number of replications</td>
</tr>
</tbody>
</table>
Estimation of antioxidant enzyme activities

Enzymes like peroxidase (POX), Catalase (CAT) and Superoxide dismutase (SOD) were extracted in suitable phosphate buffer from fully matured and freshly cut flag leaves of control and treated plants. Properly diluted crude extracts were assayed for aforesaid enzymes by following standard protocols.

Assay of enzyme Peroxidase (EC. 1.11.1.7)

Fresh and physiologically active flag leaves of fertilizer treated and control plants were collected and cut into small pieces. Accurately 100 mg of leaf samples were homogenized in pre chilled mortar and pestle, in 5 ml of 0.1 M phosphate buffer (pH 7.0). The extract was centrifuged at 4 °C in cooling centrifuge at 15000×g for 10 minutes and the supernatant was used as source of enzymes.

The peroxidase activity was assayed by (Vidyasekharan and Durairaj, 1973) method. The assay mixture of 3 ml contained 1.8 ml of 0.1 M phosphate buffer (pH 7.0), 1 ml freshly prepared 10 mM Guaiicol, 0.1 ml enzyme extract and 0.1 ml of 12.3 mM H$_2$O$_2$. Initial optical density was read at 436 nm and then increase in optical density was noted at the intervals of 30 seconds on UV-visible spectrophotometer (Shimadzu-1700). The enzyme activity was expressed as units g$^{-1}$ fresh weight.

Catalase (EC. 1.11.1.6)

Fresh and physiologically active flag leaves of fertilizer treated and control plants were collected and cut into small pieces. Accurately 100 mg of leaf samples were homogenized in pre chilled mortar and pestle, in 5 ml of 0.06 M phosphate buffer (pH 7.0). The extract was centrifuged at 4 °C in cooling centrifuge at 15000×g for 10 minutes and the supernatant was used as source of enzymes.

The catalase activity was calculated by using (Maxwell and Bateman, 1967) method. The reaction mixture contains 2.95 ml of 0.06 M phosphate buffer, 10% (w/v) H$_2$O$_2$ and 0.05 ml enzyme extract. The decrease in absorbance was measured at 240 nm by using UV-visible spectrophotometer (Shimadzu-1700). The residual H$_2$O$_2$ concentration was calculated using extinction coefficient 0.036 µM ml$^{-1}$. The enzyme activity was expressed as units g$^{-1}$ fresh weight.

Superoxide dismutase estimation (EC. 1. 15. 1. 3)

Superoxide dismutase (SOD), a metal containing enzyme plays a vital role in scavenging superoxide radical. The Superoxide dismutase activity was calculated by using (Sadasivam and Manickam, 1996). Crude enzyme extract 100 µL was mixed with a 3 ml reaction cocktail containing: 50Mm potassium phosphate buffer (pH 7.8), 13 mM methionine, 2µM riboflavin, 0.1 mM EDTA and 75 µM NBT. Final volume of reaction mixture was made equal by adding distilled water. A blank was set without enzyme and NBT to calibrate the spectrophotometer and another control was set with NBT but without enzyme as reference control. The reaction tubes were exposed to 400 W bulbs (4×100 W bulbs) for 15 minutes and immediately absorbance was taken at 560 nm. The percent inhibition was calculated. The 50% inhibition of the reaction between riboflavin and NBT in the presence of methionine is taken as 1 unit of SOD activity and the enzyme activity is expressed as units g$^{-1}$ fresh tissue.

Grain yield (kg/ha)

Samples from 1 M² area from each plot were used for Grain yield.

RESULTS AND DISCUSSION

Peroxidase (units g$^{-1}$)

The results pertaining to effect of organic fertilizers, combination of chemical fertilizer with organic fertilizers and recommended dose of NPK on leaf peroxidase 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.

Catalase (units g$^{-1}$)

The results pertaining to effect of organic fertilizers, combination of chemical fertilizer with organic fertilizers and recommended dose of NPK on leaf Catalase content in rice is given in table 3. Among the treatments the half dose of CPMR+ RDF, CPMR organic fertilizer and PMR show results at par with each other and better than CM, CMR, PM and RDF alone in both the years and even in pooled means.
Super oxide dismutase (units g\(^{-1}\))

The results pertaining to effect of organic fertilizers, combination of chemical fertilizer with organic fertilizers and recommended dose of NPK on leaf super oxide dismutase (SOD) activity in rice is given in table 3. Among the treatments the half dose of CPMR+ RDF, CPMR organic fertilizer and RDF 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 leaf Peroxidase content (units g\(^{-1}\)), Catalase content (units g\(^{-1}\)), Super Oxide Dismutase content (units g\(^{-1}\)) and grain yield in rice (O. sativa).

<table>
<thead>
<tr>
<th>Treatments (Per hectare)</th>
<th>Peroxidase (units g(^{-1}))</th>
<th>Catalase (units g(^{-1}))</th>
<th>Super Oxide Dismutase (units g(^{-1}))</th>
<th>Grain yield (Kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Pooled: 33.21</td>
<td>Pooled: 0.00</td>
<td>Pooled: 41.51</td>
<td>Pooled: 3943.90</td>
</tr>
<tr>
<td>RDF (NPK) 100:50:50 kg</td>
<td>47.30</td>
<td>42.43</td>
<td>390.37</td>
<td>35.75</td>
</tr>
<tr>
<td>CM 4t</td>
<td>37.68</td>
<td>13.46</td>
<td>338.77</td>
<td>11.35</td>
</tr>
<tr>
<td>CMR 4t</td>
<td>40.53</td>
<td>22.03</td>
<td>347.46</td>
<td>25.17</td>
</tr>
<tr>
<td>PM 4t</td>
<td>41.54</td>
<td>25.08</td>
<td>361.19</td>
<td>28.98</td>
</tr>
<tr>
<td>PMR 4t</td>
<td>44.08</td>
<td>32.73</td>
<td>413.39</td>
<td>33.82</td>
</tr>
<tr>
<td>CPMR 4t</td>
<td>45.78</td>
<td>37.85</td>
<td>420.42</td>
<td>37.18</td>
</tr>
<tr>
<td>CPMR 2t + ½ RDF</td>
<td>50.86</td>
<td>53.15</td>
<td>447.73</td>
<td>41.99</td>
</tr>
<tr>
<td>CD (0.05)</td>
<td>1.30</td>
<td>11.40</td>
<td>1.24</td>
<td>96.36</td>
</tr>
<tr>
<td>CD (0.01)</td>
<td>2.29</td>
<td>20.13</td>
<td>2.19</td>
<td>170.13</td>
</tr>
</tbody>
</table>

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 half dose of CPMR+ half dose of RDF, CPMR and PMR show results at par with each other and significantly better than CM, CMR, PM and RDF alone in both the years and even in pooled means.

Under normal conditions, plants posses scavenging systems that keep reactive oxygen species below damaging levels (Larson, 1988). When plants are subjected to environmental stresses such as drought, salinity, heat, chilling and mineral deficiency, a variety of toxic oxygen species (TOS) such as superoxide, hydrogen peroxide, hydroxyl radicals and singlet oxygen have been noted (He et al., 2011; Gorjii et al., 2011). The balance between production of reactive oxygen species and the quenching activity of the antioxidants is considered essential to avoid oxidative damage in plants (Negm et al., 2003). To prevent or alleviate injuries from activated oxygen species (AOS), plants have evolved several mechanisms that include scavenging by natural antioxidants and enzymatic antioxidants system such as superoxide dismutase, catalase and peroxidases (Scandalios, 1993). Cooperation among these enzymes is essential for the effective protection from AOS e.g., superoxide dismutases (SODs) react with superoxide radicals to produce O\(_2\) and H\(_2\)O\(_2\) whereas catalase and/or peroxidase convert H\(_2\)O\(_2\) to O\(_2\) and water (Seebba and Vitagliano, 1998).

Plants face numerous stresses in their life and the oxidative state of plants plays a pivotal role in plant defence against such stresses. Induction of various antioxidant enzymes and other defensive compounds is a common phenomenon in plants in response to different biotic and abiotic stresses (Omidi, 2010). This response occurs both in the plant organs originally attacked (local response) and in non-attacked organs (systemic response) (Metraux et al., 2002). Plants protect themselves from cytotoxic effects of aforesaid ROS with the help of antioxidant enzymes such as Peroxidase (POX), Polyphenol Oxidase (PPO), Catalase (CAT) and Superoxide Dismutase (SOD) induced in plants in response to the stress (He et al., 2011). Induced oxidative response enables the plants to cope with various kinds of stresses and allows them to be phenotypically plastic. It makes the plant more unpredictable for stress causing agents due to the variations in defence constituents of the plant (War et al., 2011a, b).
Among the ROS, \( \text{H}_2\text{O}_2 \) is a relatively stable, partially reduced form of hydrogen, diffuses freely and thus is an important factor in generation of defence responses in plants (Boka et al., 2007). A close interaction occurs between perception of \( \text{H}_2\text{O}_2 \) in response to biotic and abiotic stresses in plant systems (Syeed et al., 2011; Idrees et al., 2011). \( \text{H}_2\text{O}_2 \) acts through signal transduction pathways, which leads to the expression of defence genes (Idrees et al., 2011). Accumulation of \( \text{H}_2\text{O}_2 \) instigates a cascade of events that trigger physiological and molecular responses in plants to defend plants against biotic and abiotic stresses (War et al., 2011a, b).

Catalase is universally present oxidoreductase that decomposes \( \text{H}_2\text{O}_2 \) to water and molecular oxygen and it is one of the key enzymes involved in removal of toxic peroxides (Lin and Kao, 1999). (Sabrina et al., 2011) observed increase in activities of oxidative enzymes like peroxidase, and Catalase due fertilizers rich in phosphate in wheat plants; they showed positive correlation of antioxidant enzymes activities with levels of phosphate and MDA production in wheat plant grown under different levels of NPK fertilizers. (Whereas Malusa et al., 2002) studied role of phosphate on antioxidative system in bean plant (Phaseolus vulgaris). (Anwarul et al., 2003) observed increase in peroxidase and polyphenol oxidase in jute leaves due application of cow dung alone and in combination with NPK as well as NPK along with other minerals. (Amal et al., 2010) reported marked increase in peroxidase and catalase in sorghum plants treated with bio-fertilizers and compared to organic fertilizers. According to them, these responses may be attributed as an attempt of the plant to overcome the adverse conditions of some elements required for growth and development of sorghum plants.

Peroxidase (POX) enzyme showed increased activity with different organic fertilizers, similar observations were made by (Ahmed et al., 2010) in sunflower with Bio-N-P fertilizer and confirmed by (Kamran et al., 2006) who detected an increase in POX activity when used bacterial strains of Azotobacter to inoculate the seeds of Triticum aestivum. Moreover some investigators recorded increments in peroxidase activity as a result of plant inoculation with pseudomonas (Yi et al., 1987) on tobacco (Balamuralikrishnan et al., 2005) on sorghum bicolor and (Sardi et al., 2006) on Phaseolus vulgaris.

In present investigation SOD, POX and CAT activities showed marked increase over control plants. The increments in these enzymes might have helped the plant to destroy \( \text{H}_2\text{O}_2 \) available in normal or abnormal conditions and maintained the ascorbate pool which in turn led to elevate the plant tolerance and maintained best growth.

(Kumar et al., 2009) studied the effect of organic fertilizers on rice and observed increase in CAT, POX and SOD and PPO due to organic fertilizer treatments. According to them increase in enzyme activity may be due to interaction of available rhizobacteria and micorrhizal fungi with roots of rice fertilized with organic fertilizers.

Mineral fertilization are able to supply control plants greater amounts of nitrogen in a brief period to improve metabolism with no need of additional antioxidant enzymes, whereas in case of plants that receive the organic fertilizers face a condition of slow release of available nitrogen. That might be considered as adverse environmental conditions due to nutrient deficiency where the role of the antioxidant enzymes support plants to become more tolerant against the proposed disturbances in different plant physiological process. Soil microbes flourish and compete with plants for N and utilize organic matter for their growth; plants have some mechanism to avoid interaction of such microbes (Trinchera et al., 2008).

**CONCLUSION**

In present investigation increase in SOD, POX and CAT can be attributed to (i) interaction of VAM fungi with rice roots (ii) slow release of N responsible for nitrogen imbalance in initial growth stages and more requirement of N for growth and multiplication of microbes (iii) production of organic acid, lowering alkalinity and increase in salinity- explained on the basis of soil EC and pH and (iv) phosphate solubilization by microbes and increase in phosphate increases AO activity. In a general conclusion 2 t/ha CPMR+½RDF, CPMR and PMR organic fertilizers treatments supported better plant growth and metabolism and antioxidant enzymes, involved in detoxification , hence more productivity in rice better grain qualities were observed in these treatments.
REFERENCES


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