STUDIES ON METABOLISM OF IRON IN RICE

A Thesis
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Assam Agricultural University

In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE (*Agriculture*)

IN

AGRICULTURAL BIOCHEMISTRY

By

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MY BELOVED PARENTS
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All helps received by her have been duly acknowledged.

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CERTIFICATE – II

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ABSTRACT

Rice, though rich in carbohydrates and proteins, lacks micronutrients like iron and zinc. Considering several disadvantages of fortification of iron in food, emphasis is given on biofortification of iron in plants, including rice grains. Though, there exists threat of iron toxicity in some of the rice varieties grown in lowland acid soils of this region, the present study was conducted to know the effect of two different levels of soil iron content on some biochemical parameters including grain iron content of rice plant.

Three rice varieties, including two popular varieties of Assam, *Ranjit* and *Mahsuri,* and one traditional pigmented variety *Kajoli chakua* were cultivated in pots at two different levels of iron: marked as control and treated; in which DTPA extractable iron content of soil were 159.40 mg/kg and 182.35 mg/kg, respectively.

Within the range of soil DTPA extractable iron content (159.40 mg/kg - 182.35 mg/kg), iron toxicity was not observed. The analysis revealed that the iron content, chlorophyll content of leaves and the activities of antioxidative enzymes *viz.* peroxidase, superoxide dismutase and catalase varied significantly at different growth stages. Among the three rice varieties, uptake of iron in rice leaves and grains were found in the order *Ranjit* > *Kajoli chakua* > *Mahsuri*. The iron content of brown rice significantly differed according to its position on the rachis, the order being: top primary rachis > top secondary rachis > middle primary rachis > middle secondary rachis > bottom primary rachis > bottom secondary rachis**.** The iron content of brown rice of all the three varieties increased significantly (more than 100 % than that of control) in plants grown in soils of higher iron content. Specific activity of all the three enzymes showed that higher the iron content, more the specific activity.

Considering initial iron status of the soil, application of iron solution of suitable concentration may be advocated for increasing grain iron content of these three rice varieties.

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LIST OF ABBREVIATIONS

A.O.A.C	: Association of Official Analytical Chemist
BPR	: Bottom primary rachis
BSR	: Bottom secondary rachis
CAT	: Catalase
Cyt	: Cytochrome
DAT	: Days after transplanting
℃	: Degree centigrade
δ	: Delta
DTPA	: Diethylene Triamine Penta acetic Acid
EC	: Enzyme Commission Number
et al.	: *Et alli* (and others)
EDTA	: Ethylene Diamine Tetraacetic Acid
FIG.	: Figure
f.w	: Fresh weight
g	: Gram
ha	: Hectare
hrs	: Hours
Fe	: Iron
kg	: Kilogram
MPR	: Middle primary rachis
MSR	: Middle secondary rachis

meq	: Milliequivalent
mM	: Millimolar
min	: Minute
M	: Molar
nm	: Nanometer
N	: Nitrogen
N	: Normality
No.	: Number
ppm	: Parts per million
/	: Per
%	: Per cent
POD	: Peroxidase
P	: Phosphorus
PVP	: Polyvinyl pyrrolidone
K	: Potassium
ROS	: Reactive oxygen species
rpm	: Revolutions per minute
SE(m)	: Standard error mean
SOD	: Superoxide dismutase
TPR	: Top primary rachis
TSR	: Top secondary rachis
TEA	: Triethanolamine
Vol	: Volume

CHAPTER I

INTRODUCTION

Rice (*Oryza sativa* L.) apparently originated in South East Asia more than 6,000 years ago (Islam *et al*., 2016). In India, it is grown in about 43.79 million hectares with production of 112.91million tonnes with an average yield of 2578 kg/hectare (Anonymous, 2018).

Rice is the staple food for half of the world's population and 90% of the Asians. It is known as the 'grain of life' (Chaudhari *et al*., 2018). Rice is the major source of carbohydrate and protein. It provides around 21% of dietary energy and 15% of protein to the global population in the developing countries. It is a major source of food for more than 2.7 billion people on daily basis (Reddy *et al*., 2018). This staple food contributes up to 70% of daily calories for more than half of the world's population (Majumder *et al*., 2019).

Rice, however, is deficient in many essential micronutrients such as iron and zinc which are important for human nutrition (Sellappan *et al*., 2009).

Iron is important for people of all age groups, particularly children and pregnant women need it in more quantity (Nair and Augustine, 2018). Polished rice contains an average of only 2 mg/kg iron, whereas the recommended dietary intake of iron for human is 10-15 mg. Rice doesn't give optimum amount of micronutrients required daily, where half of the world's population (particularly Indian) is rice eating. Therefore, a slight increase in its nutritive value would be highly beneficial for alleviation of iron malnutrition and for human health. Iron deficiency is the most widespread human nutritional disorder. There are two billion anaemic people worldwide and 50% of all the anaemic cases can be attributed to iron deficiency (Krupa *et al*., 2017).

Bioavailability of iron

Iron forms present in food consist both as heme and non-heme iron. Usually, heme iron is found in meat and fish and has a high bioavailability (15%-35%); whereas, non-heme iron absorption is much lower (2%-20%) which is found

in cereals, pulses, fruit and vegetables. Non-heme iron is the major source of absorbed dietary iron in the developing countries (Abbaspour *et al*., 2014).

Many inhibitory factors in plant source food impair the absorption of iron, such as phytic acid and oxalic acid and carbonate forms an insoluble salt with iron. (Meng *et al*.; 2005). In most cereals, approximately 80% of the total phytic acid (FIG. 1.1.1) gets accumulated in the aleurone layer of the grains. Phytic acid accumulates as mixed salts called phytate (Majumder *et al*., 2019). Phytate is composed of a phosphorylated *myo*-inositol ring and strongly chelates metal cations, including iron, zinc and manganese (Morrissey and Guerinot, 2009). However, the enzyme phytase, which hydrolyses phytic acid can increase the bioavailability of iron in rice and improve iron nutrition. Although trace amounts of phytic acid strongly inhibit iron absorption, insertion of phytase gene into rice has great potential for improving the iron nutrition in rice-eating populations. Cysteine and cysteine containing peptides together with vitamin C enhance the absorption of non-heme iron in man (Meng *et al*.; 2005).

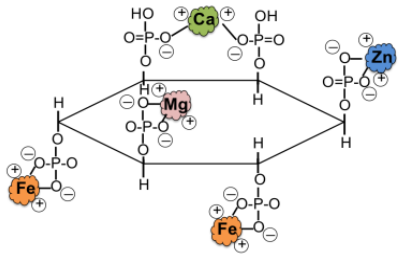


FIG. 1.1.1. PHYTIC ACID AS CHELATOR OF DIVALENT CATION OF IRON (Fe^{2+}) (Majumder *et al*., 2019)

Importance of iron in plants

Iron is an essential element for all plants. Iron is the third most limiting nutrient for plant growth and metabolism. It has 2 forms- Ferric (Fe^{3+}) and Ferrous (Fe^{2+}). Fe^{3+} is insoluble and its uptake is difficult whereas, Fe^{2+} is soluble and readily available to plants (Santos *et al*., 2017). It has many important biological roles in photosynthesis, chloroplast development and chlorophyll biosynthesis. It is a major constituent of the cell redox systems like heme proteins including cytochromes, catalase, peroxidase, Fe-S proteins like ferredoxin, aconitase and superoxide dismutase (Baruah and Bharali, 2015).

Iron uptake and transport in rice

Iron is required by the plants throughout its growth period.

The growth period of the rice plant (FIG. 1.1.2.) is divided into three stages :

1. Vegetative (germination to panicle initiation)
2. Reproductive (panicle initiation to flowering)
3. Ripening (flowering to mature grain)

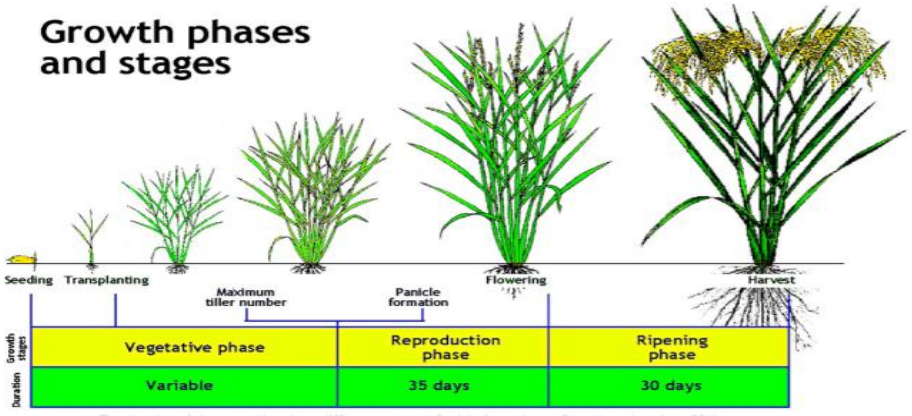


FIG. 1.1.2. GROWTH STAGES OF RICE PLANT (Anonymous, 2015)

In xylem, iron is mainly complexed to citrate for long distance circulation of iron chelates. In phloem, it is likely to be chelated to a small plant and fungi-specific metabolite, nicotianamine (NA). Transloading iron from xylem to phloem requires Fe-NA-specific transporters. Chlorosis results from the proteolytic loss of photosystems and cyt b6/f complex (Briat *et al.*, 2007).

Iron moves to the seeds *via* the phloem, as the flow of the xylem is driven by transpiration and seeds do not transpire. Developing seeds receives iron from the roots and from senescent leaves. Rice transports only 4% of shoot iron to the seeds (Morrissey and Guerinot, 2009). The flag leaves are the main source of photoassimilates for the development of rice seeds. During the reproductive development in rice, the iron content of the flag leaf decreases and the iron content of grain increases (Santos *et al*., 2017).

Iron content of different forms of rice

Rice iron concentration becomes drastically reduced more than any other mineral due to post-harvest processing. Paddy (rough rice) contains 38 ppm

(mg/kg) of iron that is reduced to 8.8 ppm (mg/kg) in brown rice after processing and finally 4.1 ppm (mg/kg) in milled rice (Majumder *et al*., 2019).

Krupa *et al*., (2017) reported that the highest iron content was found in paddy (grain with husk) compared to brown rice (after removing husk). The iron content found in paddy ranged from 1.11-1.77 mg/100g; whereas, the iron content found in brown rice ranged from 0.74-1.15 mg/100g. Brown rice was subjected to different levels (5% and 10%) of polishing. Loss of iron was found in both the levels of polishing. Polishing removes almost all the aleurone and embryo, which is the main storehouse for major micronutrients. Percent loss of iron was found more in 10% polishing than in 5% polishing. Most of the iron in rice is lost during processing.

Iron deficiency in plants

Rout and Sahoo, (2015) reported that iron is a constituent of non-heme iron protein that is involved in photosynthesis, nitrogen fixation and respiration. Limitation of iron causes the decrease of many photosynthetic components, including the Fe-S protein ferredoxin. It causes alterations in root morphology. Symptoms of iron deficiency includes interveinal chlorosis (yellowing of leaf) in young leaves as well as in poor root formation. When iron deficiency is severe, it leads to growth retardation, stasis and death.

Iron toxicity

Iron toxicity is a major nutrient disorder affecting rice production of wetland rice in the irrigated and rain fed ecosystem. Symptoms of iron toxicity are leaf bronzing, stunted growth, low germination rates, etc.

Iron is toxic when high level accumulates in plant. It can act catalytically *via* the Fenton reaction to generate hydroxyl radicals, which can damage lipids, proteins and DNA (Rout and Sahoo, 2015) as shown in FIG. 1.1.3.

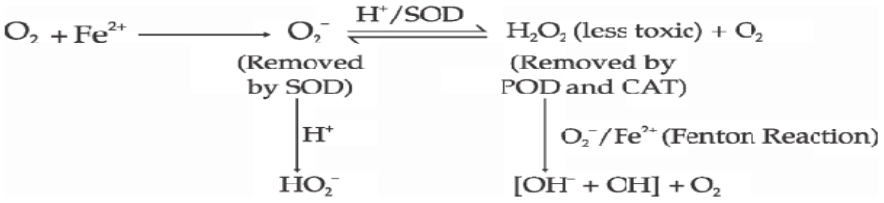


FIG. 1.1.3. FENTON REACTION (Baruah and Bharali, 2015)

Lowland rice frequently suffers from iron toxicity. Overloading of iron in rice plants shows tiny brown spots starting from the tips, spreading towards the base of lower leaves (Saikia and Baruah, 2012).

Iron toxicity is reported to be an important yield limiting factor in terms of productivity. Iron toxicity in rice has been reported in several countries in Asia, South America, West and Central Africa and Uganda (Sahrawat, 2004). Injuries due to iron toxicity are estimated to reduce overall rice grain yield by 30-60 per cent (Sahrawat, 2000 and Majerus *et al*., 2007). Depending on the region, the soil type, the cropping season, and the severity and duration of Fe-toxicity occurrence, rice genotypes strongly differ in their response patterns to excess amount of Fe^{2+}. Hence, the toxicity impact can be reduced by using Fe tolerant genotypes, soil, water and nutrient management practices. Cultivars that reportedly showed Fe^{2+} tolerance in soil-environmental conditions of one region frequently submit to iron toxicity in another region (Baruah and Bharali, 2015).

Nutritional disorders associated with iron toxicity have been divided into direct and indirect toxicity (Backer *et al*., 2005).

Direct toxicity is related to the plant's excessive iron absorption, which damages the plant cells. Symptoms appear initially on younger leaves, where the element concentrates in small brown dots. This phenomenon is known as bronzing (Baruah and Nath, 1996).

Indirect toxicity results from the limited absorption of several nutrients such as calcium, magnesium, potassium, phosphorus and iron itself, due to iron precipitation on rice root epidermis. The formation of an oxide-hydroxide Fe^{3+} layer on the root blocks nutrient absorption, resulting in multiple nutritional deficiencies (Baruah and Bharali, 2015).

Iron toxicity is characterized by "bronzing" or "yellowing" of oldest leaves and formation of ROS in cells which affects the synthesis of chlorophyll, protein, leaf free amino acid and nitrate reductase activity. Higher Fe uptake by plants is reported to reduce the protein synthesis in the leaf (Baruah *et al*., 2007; Silveira *et al*., 2007; Saikia and Baruah, 2012). Iron induced detrimental effects may be the reason of lower chlorophyll content in iron sensitive varieties (Baruah and Bharali, 2015).

Phytoferritin and iron toxicity

In most organisms, including plants, there is an iron storage protein-ferritin (Theil, 1987). The iron present in ferritin cannot be made unavailable by phytic acid as it is surrounded by protein. Ferritin is also known for development of immunity. Though, ferritin was reported to store 92% of the total Fe indicating positive correlation between Ferritin and iron content in rice grain, some deviations were observed among rice genotypes, suggesting that it is not always that high ferritin leads total increase of iron content in rice grain (Pandey *et al*., 2018). In multiple experiments involving ferritin gene incorporation, endosperm specific promoters were used for *ferritin* gene expression in rice, resulting up to 3.7 fold iron increase in rice grain (Majumder *et al*., 2019).

Ferritin is considered crucial for iron homeostasis. It consists of multimeric spherical protein called phytoferritin, able to store upto 4500 Fe atoms inside its cavity in non toxic form (Briat *et al*., 2006, 2007). It functions as a cellular Fe buffer. Plant ferritin is found mainly in plastids and also in mitochondria (Zancani *et al*., 2007). It is synthesized in response to various environmental stresses including photo inhibition and iron overloading (Murgia *et al*., 2001, 2002). The main function of plant ferritin is not to store and release iron, but to scavage free reactive iron and prevent oxidative damage (Ravet *et al*., 2009). A resistant variety may accumulate more amount of phytoferritin which forms complex with Fe, reducing iron toxicity damage (Baruah and Bharali, 2015).

Plants tightly control iron homeostasis and react to iron deficiency as well as iron overload. The ability of plants to respond to iron availability affects human nutrition, both in terms of crop yield and the iron concentration of edible tissues (Morrissey and Guerinot, 2009).

Soil environment and iron toxicity

The iron content in soil varies from 1% to 20%, averaging 3.2%. Its normal concentration in plants is only 0.005%. Its main cause is that iron in the soil exist mostly in the forms of hydrogen oxide, oxide, phosphate or other deposited compounds (Meng *et al*., 2005). Plants acquire iron from the rhizosphere. Although iron is one of the most abundant metal in the earth's crust, its availability to plant roots is very low. Its availability is dictated by the soil redox potential and pH. In

aerobic soils (high pH), iron is readily oxidized and is in the form of insoluble ferric oxides. In anaerobic conditions (low pH), ferric iron is reduced to ferrous iron and becomes more available for uptake by roots. Plants solubilizes ferric ions and make them available for absorption (Morrissey and Guerinot, 2009).

Baruah and Bharali, 2015 stated that the severity of iron-toxicity expression in rice has been related to a number of soil factors. These involve most prominently (1) the content and type of the clay minerals (2) the amount of exchangeable soil Fe (3) the soil pH, and (4) the presence of "stress factors". They referred to the study of Das *et al*., 1997 which stated that the concentration of soil Fe^{2+} was reportedly less in clay than in sandy soils. Clay content affected the Fe dynamics primarily *via* Fe retention on clay-mineral surfaces due to cation-exchange capacity. Baruah and Bharali, 2015 also stated about large inconsistency within the published literature on iron toxicity with regard to soil Fe content ranging from 20–5000 mg $kg^{-1}$and leaf Fe concentrations (300–2000 mg kg^{-1}), time of toxicity occurrence-from 2 weeks after rice transplanting to the late reproductive phase, the distribution of toxicity symptoms in the field, and the observed yield loss.

Iron metabolism and role of anti-oxidative enzymes

Plant cells employ their antioxidant defense system like peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) to protect the tissues from iron mediated oxidative damage. However, iron is one of the constituents for all these three enzymes (Baruah and Bharali, 2015).

POD is an important antioxidant enzyme involved in response to environmental stress. It reduces toxic effect of H_2O_2 and severity of oxidative stress. SOD is the antioxidant enzyme in aerobic cells responsible for reduction of reactive oxygen species. SOD activity determines the concentration of O_2 and H_2O_2. The metal cofactors are in oxidized form (M^{n+1}) which catalyzes the dismutation of superoxide radical to produce molecular O_2 and H_2O_2. To overcome this, plant cells develop enzymatic mechanism to reduce the damaging effects by Fenton reaction. CATs are tetrameric heme containing enzymes with the potential to directly dismutate H_2O_2 into H_2O and O_2.. The CAT activities increase significantly by heavy metal stress. CAT activities also play a role in iron tolerance in rice (Saikia and Baruah, 2012).

To manage ROS outbreaks, plants have evolved intricate antioxidant defense systems, consisting of detoxifying or ROS scavenging enzymatic antioxidants, *viz.* peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and non-enzymatic antioxidants *viz.* glutathione, ascorbic acid, carotenoids, α-tocopherol and flavonoids (Poli *et al*., 2018).

Iron biofortification

Kok *et al*., (2018) stated that iron biofortification, the process of improving the bioavailability of iron in food crops can be achieved *via* agronomic practices, conventional breeding, and genetic engineering. Biofortification through agronomic practices can be performed through fertilizer or foliar feeding. Agronomic biofortification is a traditional biofortification approach, which involves micronutrient uptake from the surrounding soil and translocation into the edible parts of the plants. In addition, macronutrient also plays a crucial role in iron biofortification in plants. It is also emphasized that combined application of both NPK fertilizer and iron fertilizer could be a potential approach to increase iron bioavailability in rice through root development, shoot transport and re-localization, which improved the translocation of iron into rice grain.

International Rice Research Institute (IRRI) began to examine the effect of certain soil characteristics on iron content in crop and the research on iron content of rice has been one of the global research hot issues (Meng *et al*., 2005). It is thought that the practice of increasing soil iron content to a level at which it might lead to improve iron content of edible portion of brown rice together with eliminating iron toxicity symptoms, might be a promising approach towards biofortification of iron in rice.

Considering the availability of limited information on the soil iron content and its relationship with iron content of brown rice and the activities of few iron containing antioxidative enzymes, the present investigation has been proposed with the following objectives:

1. To know the effect of soil iron content on some important biochemical components including the iron content of grain of rice and its behaviour on crop growth

2. To know the activities of a few oxidative enzymes related to iron metabolism as influenced by soil DTPA (Diethylene triamine pentaacetic acid) extractable iron content.

CHAPTER II

REVIEW OF LITERATURE

The literatures related to the present investigation "Studies on metabolism of iron in rice" are presented in this chapter.

2.1. Moisture content

2.1.1. Moisture content of rice leaves

Tang *et al*., (2020) reported that the moisture content of rice leaves ranged between 26.39% to 60.71%, on fresh weight basis.

2.1.2 Moisture content of paddy

Davis, (1944) reported that the optimum harvest moisture content for the paddy of the *Caloro* variety was 20-24%.

Pominski *et al*., (1961) showed that paddy moisture content had significant effect on milling yields and concluded that for each 1% decrease in moisture content, head yields and total yields increased 3% and 0.7%, respectively within the paddy moisture content range of 10-14%.

The range of moisture content of paddy at harvesting is 20%-25%. If the grain id too dry, it will lead to shattering. If the grain is too wet, there will be unfilled and many green grains (www.knowledgebank.irri.org).

2.1.3. Moisture content of rice

Pathak (2008) reported that the moisture content of some hill rice cultivars of Assam ranged from 8.29% - 10.10%.

Oko and Ugwu, (2011) reported that the moisture content of some rice varieties of South-Eastern Nigeria ranged between 3.67% - 18.00%.

Mbatchou and Dawda, (2013) reported that the moisture content of milled rice samples ranged from 8.50% - 22.00%.

Dutta *et al*., (2015) reported that the moisture content of some rice varieties of

Assam like *Ranjit, Kala chakua, Aghuni bora and Bhogali bora* to be 13.1%, 12.9%, 13.1% and 13.2%, respectively.

Pathak (2015) stated that the moisture content of some glutinous rice cultivars of Assam ranged from 8.33% - 10.74%.

Das *et al*., (2018) reported that the moisture content of some brown form of *chakua* rice varieties of Assam ranged between 10.1% - 11.8%, on fresh weight basis.

Chatterjee and Das, (2019) studied the chemical properties of six indigenous rice varieties of Assam and reported that the moisture content of unpolished rice ranged between 12.25% - 15.50%, on fresh weight basis.

Bhattacharjee and Das, (2020) reported that the moisture content of brown forms of speciality rice varieties of Assam ranged between 11.51% - 14.05%, on fresh weight basis.

2.2. Chlorophyll content of rice leaf

Baruah and Nath, (1996) studied the effect of different soil iron levels on chlorophyll content (fresh weight basis) of leaves in 6 varieties of rice and reported that it ranged between 2.227 mg/g – 2.870 mg/g, 1.527 mg/g – 1.920 mg/g and 1.120 mg/g – 1.773 mg/g for control, 100 ppm and 200 ppm iron solutions, respectively.

Chutia and Borah, (2012) reported that the total chlorophyll content of leaves of 12 different traditional rice genotypes grown under three different water regimes ranged between 2.28 to 20.13 mg/g (control), 1.89 to 14.10 mg/g (upland) and 1.29 to 17.07 mg/g (potted).

Saikia and Bhuyan, (2017) reported that the chlorophyll content (fresh weight basis) of rice leaves for different levels of iron (control, 100 ppm, 200 ppm and 300 ppm solution) ranged from 1.9 to 3.7 mg/g, 2.3to 3.0 mg/g, 2.8 mg/g to 3.0 mg/g and 2.2 to 2.4 mg/g at maximum tillering stage and 2.2 to 3.8 mg/g, 2.8 to 4.8 mg/g, 2.2 mg/g to 4.5 mg/g and 1.3 to 4.4 mg/g at grain filling stage, respectively.

2.3. Ash content

2.3.1. Ash content of rice leaves

Vadiveloo, (2000) reported that the ash content of rice straw was found to be 18.5 %, on dry weight basis.

2.3.2. Ash content of brown rice

Zubair *et al*., (2012) reported that the ash content of dehulled rice grain of some selected rice varieties of Pakistan ranged between 1.48% - 1.98%, on dry weight basis.

Pathak (2015) stated that the ash content of some glutinous rice cultivars of Assam ranged from 1.00% - 2.00%.

Das *et al*., (2018) reported that the ash content of some brown form of *chakua* rice varieties of Assam ranged between 0.66% - 1.52%, on dry weight basis.

Mudoi and Das, (2018) reported that the ash content of brown rice of twenty one indigenous red grain cultivars of Assam ranged between 0.73% - 1.85%, on dry weight basis.

Bhattacharjee and Das, (2020) reported that the ash content of brown forms of speciality rice varieties of Assam ranged between 0.66% - 1.00%, on dry weight basis.

2.3.3. Ash content of rice husk

Natarajan and Ganapathy, (2009) studied the pyrolysis of rice husk in a fixed bed reactor and reported that the ash content of husk of Indian rice variety to be 17.09% at 9.45 % moisture content.

Singh *et al*., (2013) reported the ash content of husk of Indian rice variety to be 9.29% at 4.65% moisture content.

2.4. Iron content of rice

2.4.1. Iron content of rice leaves

Nandi, (1997) reported about the iron content (dry weight basis) of rice plant as influenced by different levels of ferrous iron (100-500 ppm) and it ranged between 32.03 mg/100g – 34.77 mg/100g, 31.10 mg/100g – 34.07 mg/100g

and 26.62 mg/100g – 29.96 mg/100g at 30 days after transplanting (DAT), 60 DAT and at harvest, respectively.

Das *et al*., (1997) reported the iron content (dry weight basis) of rice (variety *Mahsuri*) leaves to be 629.8 ppm (62.98 mg/100g) and 597.8 ppm (59.78 mg/100g) in clay soil, while 665.6 ppm (66.56 mg/100g) and 633.3 ppm (63.33 mg/100g) in sandy clay loam soil at maximum tillering stage and at harvest, respectively.

Borah *et al*., (2000) reported that the iron content (dry weight basis) of rice leaves (variety *Mahsuri*) ranged from 61 - 82 mg/kg to 308 - 315 mg/kg and 158 - 165 mg/kg to 334 - 342 mg/kg at maximum tillering stage; 52 - 73 mg/kg to 253 - 269 mg/kg and 104 - 112 mg/kg to 248 - 260 mg/kg at harvest, grown in soils of low initial iron content (DTPA-Fe 109 ppm) and high initial iron content (DTPA-Fe 164 ppm), respectively.

2.4.2. Iron content of rice

Gregorio *et al*., (2000) reported that the iron content of some selected brown rice varieties ranged between 1.18 mg/100g to 2.24 mg/100g, on dry weight basis.

Kennedy and Burlingame, (2003) reported that the iron content of 24 selected rice varieties grown in greenhouse condition which ranged between 1.10 mg/100g – 2.64 mg/100g, on dry weight basis.

Meng *et al*., (2005) studied the iron content and bioavailability of iron in rice and reported that the iron content in brown rice ranged between 0.2 mg/100g – 5.2 mg/100g, on 14% moisture content.

Deepa *et al*., (2008) reported that the iron content (dry weight basis) of rice varieties *Njavara*, *Jyothi* and *IR64* were 1.93 mg/100g, 3.95 mg/100g and 2.73 mg/100g, respectively.

Anuradha *et al*., (2012) analyzed 126 brown rice accessions and reported the iron content to be 6.2 ppm (0.62 mg/100g) to 71.6 ppm (7.16 mg/100g), on dry weight basis.

Sharma *et al*., (2012) worked on six red rice varieties of Himachal Pradesh. They reported that the iron content ranged between 0.029 mg/100g – 0.063 mg/100g, on dry weight basis.

Thongbam *et al*., (2012) worked on eighteen indigenous rice varieties of Tripura and reported that the iron content ranged between 2.32 mg/100g – 15.42 mg/100g.

Zubair *et al*., (2012) reported that the iron content of dehulled rice grain of some selected rice varieties of Pakistan ranged between 18.6 mg/100g – 30.1 mg/100g.

Shahid *et al*., (2014) reported that the iron content of grain of four prominent rice cultivars *viz*. *Naveen, Lalat, Sebati, Pusa-44*, widely grown across Orissa to be 10.1mg/100g, 11.8 mg/100g, 10.3 mg/100g and 12.4 mg/100g, respectively.

Su *et al*., (2014) reported about the variations of the iron content of grain according to the position in the panicle. They reported that the concentration of iron in six *japonica* rice genotypes ranged between 0.968 mg/100g to 1.193 mg/100g, 0.926 mg/100g to 1.142 mg/100g, 0.914 mg/100g to 1.133 mg/100g, 0.881 mg/100g to 1.092 mg/100g, 0.840 mg/100g to 1.042 mg/100g and 0.818 mg/100g to 1.008 mg/100g in top primary rachis, top secondary rachis, middle primary rachis, middle secondary rachis, bottom primary rachis and bottom secondary rachis, respectively.

Pathak, (2015) stated that the iron content of some glutinous rice cultivars of Assam ranged from 2.38 mg/100g – 4.20 mg/100g.

Kumar *et al*., (2017) reported that the iron content of six selected rice varieties ranged between 12.48 mg/100g – 24.15 mg/100g.

Das *et al*., (2018) reported that the iron content of brown rice of seventeen *chakua* (intermediate amylose containing) varieties of Assam ranged from 1.04 mg/100g – 643.5 mg/100g, on dry weight basis. The iron content of two *chakua* rice varieties '*Kajoli chakua*' and '*Saru chakua*' were found to be remarkably higher, 643 mg per 100g (almost 50% of total minerals) and 44.97mg per 100g, respectively. The highly intense colour of the brown form of this variety justifies the vernacular name of the variety 'Kagoli chakua', which means red colour.

Chatterjee and Das, (2019) studied the chemical properties of six indigenous rice varieties of Assam and reported that the iron content of unpolished rice ranged between 1.54 mg/100g – 4.68 mg/100g.

Mudoi and Das, (2019) reported that the iron content of few indigenous red rice germplasm (brown form) of Assam ranged between 2.12 mg/100g – 54.40 mg/100g.

2.4.3. Iron content of rice husk

Meng *et al*., (2005) studied the iron content and bioavailability of iron in rice and reported that the iron content in rice husk ranged between 3.9 mg/100g – 9.5 mg/100g, on fresh weight (14 % moisture level) basis.

2.6. Peroxidase (POD)

2.6.1. Total activity of peroxidase

Saikia and Baruah, (2012) reported the total peroxidase activity in leaf tissues in three lowland rice cultivars of Assam. They reported the total activity to be higher in all the varieties irrespective of Fe^{2+} concentration in the medium. A significant induction of POD activity was found in *Ranjit* and *Siyal Sali* at higher level of Fe^{2+} in the medium. The POD activity in *Mahsuri* did not change at higher levels of Fe^{2+} in the medium. They suggested that the lower POD activity in *Mahsuri* compared to other two varieties might be due to lower Fe^{2+} uptake or translocation of less Fe^{2+} from roots to leaves.

2.6.2. Specific activity of peroxidase

Rossatto, *et al*., (2017) studied the specific activity of peroxidase in leaves of rice variety *BRS AG* (cultivated for ethanol production and animal feed utilisation) in both control and salt (NaCl) stressed condition (136 mM). They reported that the specific activity of peroxidase was higher (2.4 μmol/min/mg protein) in stressed condition than in control (1.2 μmol/min/mg protein) at 10 days after germination. At 15 and 20 days after germination, the same was observed to be higher in control than in stressed condition.

Poli *et al*., (2018) reported about the peroxidase activity in leaves in normal and low P conditions of soil. The peroxidase activity at vegetative stage was found to be 2 units/mg protein and 4 units/mg protein, respectively in high yielding

mutants and 2 units/mg protein and 13 units/mg protein, respectively in low yielding mutants. They also reported the same at reproductive stage to be 2 units/mg protein and 23 units/mg protein, respectively in high yielding mutants and 2 units/mg protein and 26 units/mg protein, respectively in low yielding mutants.

2.7. Superoxide dismutase (SOD)

2.7.1. Total activity of superoxide dismutase

Saikia and Baruah, (2012) reported the total activity of superoxide dismutase in leaf tissues in three lowland rice cultivars of Assam. They reported that the varieties *Ranjit* and *Siyal Sali* maintined a higher SOD activity compared to *Mahsuri* in controlled soil. With the increment of Fe^{2+} concentration the varieties *Ranjit* and *Siyal Sali* recorded a sharp decrease in SOD activity. The decrease of SOD activity might be due to enzyme inhibition, as higher iron is likely to inhibit the enzyme.

2.7.2. Specific activity of superoxide dismutase

Rossatto, *et al*., (2017) studied the specific activity of superoxide dismutase in leaves of rice variety *BRS AG* in both control and salt (NaCl) stressed condition (136 mM). They reported that the specific activity of superoxide dismutase was lower in control (12 units/mg protein) than in stressed condition (25 units/mg protein) at 10 days after germination and 9 units/mg protein in control and 22 units/mg protein in stressed condition at 15 days after germination. At 20 days after germination, the same was observed to be higher (36 units/mg protein) in control than (30 units/mg protein) in stressed condition.

Poli *et al*., (2018) reported about the superoxide dismutase activity in rice leaves in both normal and low P conditions of soil. The superoxide dismutase activity at vegetative stage was found to be 2.9 units/mg protein and 8.8 units/mg protein, respectively in high yielding mutants and 2.2 units/mg protein and 5.4 units/mg protein, respectively in low yielding mutants. They also reported the same at reproductive stage to be 2.2 units/mg protein and 6.6 units/mg protein, respectively in high yielding mutants and 3.4 units/mg protein and 7.8 units/mg protein, respectively in low yielding mutants.

2.8. Catalase (CAT)

2.8.1. Total activity of catalase

Saikia and Baruah, (2012) reported the total activity of catalase in leaf tissues in three lowland rice cultivars of Assam. They reported variations in the total activity of the enzyme for the three rice varieties, both for control and treated conditions. Varieties *Siyal Sali* and *Ranjit* recorded a decreasing trend of catalase activity under higher iron concentrations and suggested that reduction of catalase activity in *Ranjit* and *Siyal Sali* was due to acceleration of Fenton reaction in forward reaction.

2.8.2. Specific activity of catalase

Rossatto, *et al*., (2017) studied the specific activity of catalase in leaves of rice variety *BRS AG* in both control and salt (NaCl) stressed condition (136 mM). They reported that the specific activity of catalase was higher in control (2.4 μmol/min/mg protein) than in stressed condition (0.18 μmol/min/mg protein) at 10 days after germination. The same was observed to be 0.28 μmol/min/mg protein and 0.34 μmol/min/mg protein in control and 0.30 μmol/min/mg protein and 0.36 μmol/min/mg protein in stressed condition at 15 and 20 days after germination, respectively.

Poli *et al*., (2018) reported about the catalase activity in rice leaves for both normal and low P conditions of soil. The catalase activity at vegetative stage was found to be 2.2 units/mg protein and 8.6 units/mg protein, respectively in high yielding mutants and 2 units/mg protein and 5.8 units/mg protein, respectively in low yielding mutants. They also reported the same at reproductive stage to be 2 units/mg protein and 6 units/mg protein, respectively in high yielding mutants and 2 units/mg protein and 5.6 units/mg protein, respectively in low yielding mutants.

2.9. Phytic acid in rice

Liu *et al*., (2005a) reported that for 72 *Japonica* rice cultivars of China, phytic acid content ranged from 685 mg/100g to 1030 mg/100g.

Liu *et al*., (2005b) worked on positional variation in phytic acid content within panicle using six different *Japonica* rice cultivars and concluded that phytic acid content ranged between 642 mg/100g to 1021 mg/100g.

Wei *et al*., (2007) reported that the phytic acid content of twenty-nine *Japonica* rice varieties grown in China ranged between 699 mg/100g - 1034 mg/100g.

Magdy *et al*., (2010) reported that the phytic acid content of white (milled)

and brown rice *viz*. white rice with high amylose, white rice with low amylose, brown rice with high amylose and brown rice with low amylose to be 411.25 mg/100g, 522.25 mg/100g, 551.0 mg/100 g and 750.0 mg/100g, respectively.

Su *et al*., (2014) worked on positional variation in grain mineral nutrients within a panicle using six different *Japonica* rice cultivars and their relation to phytic acid concentration. They reported that the phytic acid content ranged from 368 mg/100g to 613 mg/100g.

Bhattacharjee, (2019) found the phytic acid content of brown forms of speciality rice varieties of Assam to be 274.20 mg/100g to 345.92 mg/100g.

to be 303.46 mg/100g.

2.10. Soil pH

Das *et al*., (1997) reported the pH to be 5.2 and 4.9 for clay soils of Nagaon district, Assam with low (DTPA-Fe 112.5 mg/kg) and high initial iron content (DTPA-Fe 163.5 mg/kg), respectively.

Borah *et al*., (2000) reported the pH to be 5.1 and 4.9 for clay soils of Nagaon district, Assam with low (DTPA-Fe 109 mg/kg) and high initial iron content (DTPA-Fe 164 mg/kg), respectively.

Bhuyan *et al*., (2014) reported the pH to be 4.60 to 6.61 for soils of Lakhimpur district, Assam with DTPA-Fe 36.4mg/kg to 224.0 mg/kg.

2.11. Soil organic carbon

Das *et al*., (1997) reported the soil organic carbon to be 12.30 g/kg for soils with low initial iron content (DTPA-Fe 112.5 mg/kg) and 13.40 g/kg for soils with high initial iron content (DTPA-Fe 163.5 mg/kg).

Borah *et al*., (2000) reported the soil organic carbon to be 10.60 g/kg for soils with low initial iron content (DTPA-Fe 109 mg/kg) and 12.60 g/kg for soils with high initial iron content (DTPA-Fe 164 mg/kg).

Bhuyan *et al*., (2014) reported the soil organic carbon to be 1.20 g/kg to 18.30 g/kg for DTPA-Fe content 36.4 to 224 mg/kg.

2.12. Cation exchange capacity of soil

Das *et al*., (1997) reported the cation exchange capacity of soil to be 18.6 cmol (p^+) kg^{-1} for soils with low initial iron content (DTPA-Fe 112.5 mg/kg) and 18.4 cmol (p^+) kg^{-1} for soils with high initial iron content (DTPA-Fe 163.5 mg/kg).

Borah *et al*., (2000) reported the cation exchange capacity of soil to be 17.2 cmol (p^+) kg^{-1} for soils with low initial iron content (DTPA-Fe 109 mg/kg), 18.4 cmol (p^+) kg^{-1} for soils with high initial iron content (DTPA-Fe 164 mg/kg).

Bhuyan *et al*., (2014) reported the cation exchange capacity of soil to be 3.30 cmol (p^+) kg^{-1} to 14.00 cmol (p^+) kg^{-1} for DTPA-Fe 36.4 to 224 mg/kg, respectively.

2.13. DTPA extractable iron of soil

Das *et al*., (1997) reported the DTPA extractable iron for soils of Nagaon district of Assam to be 112.5 mg/kg to 163.5 mg/kg.

Borah *et al*., (2000) reported the DTPA extractable iron for soils of Nagaon district of Assam to be 109 mg/kg to 164 mg/kg.

Bhuyan *et al*., (2014) reported the DTPA extractable iron for soils of Lakhimpur district of Assam to be 36.4 mg/kg to 224 mg/kg.

CHAPTER III

MATERIALS AND METHODS

The present investigation "Studies on metabolism of iron in rice" was conducted at the Department of Biochemistry and Agricultural Chemistry, Assam Agricultural University, Jorhat. The materials used and the methods followed in the present investigation are described in this chapter.

3.1. Materials

3.1.1. Crop varieties

Three rice varieties *Ranjit*, *Mahsuri* and *Kajoli chakua* were collected from Regional Agricultural Research Station (RARS), Titabar, Assam Agricultural University, Jorhat, Assam. The details of the varieties are presented in Table 3.1.1.

Table 3.1.1. The details of the rice varieties used in the present study

Sl. No.	Varieties	Characteristics	Date of sowing	Date of transplanting
1.	*Ranjit*	High yielding semi dwarf variety; short, fine quality grain	27th June, 2019	29th July, 2019
2.	*Mahsuri*	High yielding tall variety; medium, slender grain quality	27th June, 2019	29th July, 2019
3.	*Kajoli chakua*	Traditional *chakua* variety	27th June, 2019	29th July, 2019

3.1.2. Germination test

Sixty seeds of each variety were germinated on wet filter paper kept over petri plates. There was 100% germination for all the three rice varieties.

3.1.3. Collection, preparation of soil sample and preparation of pots

Bulk surface soil sample (0-20 cm depth), classified as clay was collected from rice growing areas of Instructional-cum-Research (ICR) farm of Assam Agricultural University, Jorhat, Assam. The collected soil samples were air dried under sun before putting in the pots.

Plastic pots of 15 kg capacity (24 cm diameter and 30 cm depth) were used for growing the rice plants. Six kg of well prepared soil was put into each of the plastic pots for the experiment.

3.1.4. Application of fertilizer and nutrients in soil

Each pot was supplied with 40 g FYM and was mixed with the soil. N, P_2O_5 and K_2O fertilizer @ 0.30 : 0.60 : 0.30 g/pot in the form of urea, single super phosphate and muriate of potash, respectively were mixed with the soil and applied four days before transplanting. The pots were divided into two sets based on soil iron content. The soil of the pots of the control set contained no added iron, instead 1 L distilled water, added two days before transplanting. The soil of pots of the treated set contained added iron. Two days before transplanting of seedlings, to the soils of each of the treated pots, 1 L solution of 50 ppm iron (50 mg/L) in the form of Ferrous sulphate ($FeSO_4.7H_2O$) was added, which was prepared using distilled water. The soil of the pots was puddled after addition of water and iron solution. Representative soil samples from both control and treated pots were collected at this stage for further analysis of various soil characteristics before plant growth. Three rice seedlings (30 days old) were transplanted in each pot. The soil was submerged up to a depth of 5 cm with distilled water till the grain filling stage.

3.1.5. Collection of leaf and grain samples for analysis

The leaf and grain samples were collected as mentioned below:

Sl. No.	**Stages**	**Days after sowing**		
		Ranjit	*Mahsuri*	*Kajoli chakua*
1.	Maximum tillering stage	67	65	70
2.	Grain filling stage	144	142	147
3.	Harvesting stage	174	172	176

After harvesting, the grain samples were dried and dehusked. Chlorophyll content and phytic acid P content were analysed only in leaves and brown rice, respectively. The other parameters were analysed both in leaves and brown rice. However, the rice husk too, of the respective varieties were analysed for ash and iron content.

The brown rice samples, for estimation of ash and iron content at harvesting stage were analysed for different positions of grain within a rice panicle *viz*. top primary rachis, top secondary rachis, middle primary rachis, middle secondary rachis, bottom primary rachis and bottom secondary rachis as shown in FIG. 3.1.

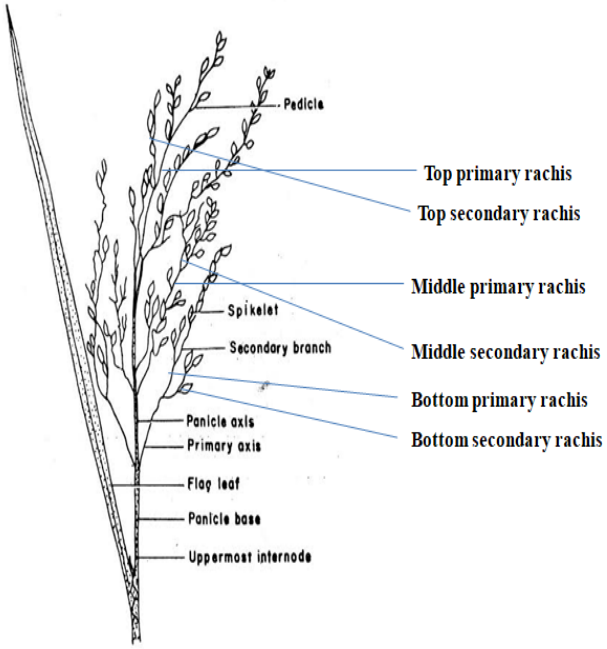
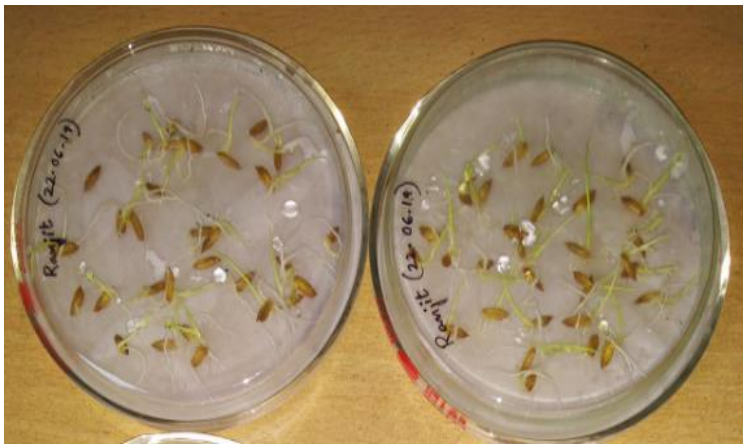
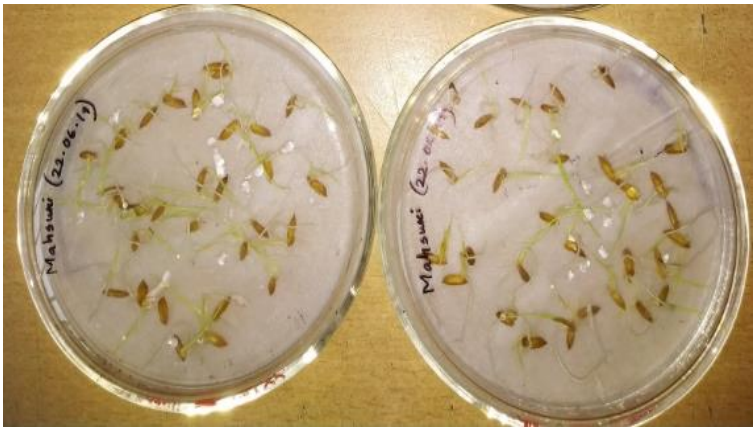


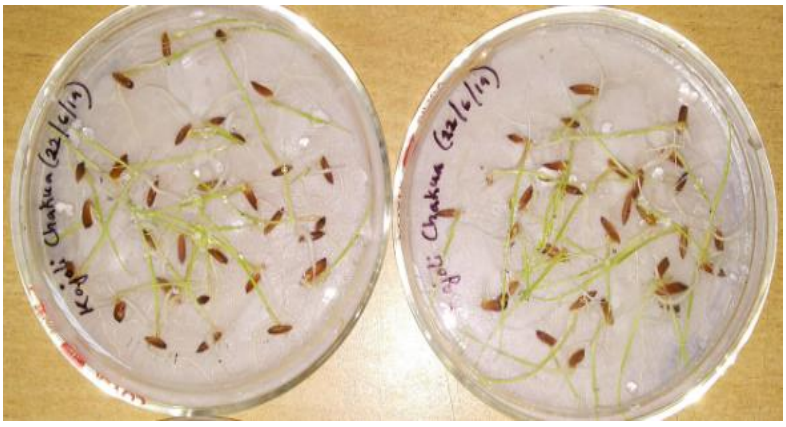
FIG. 3.1. POSITION OF GRAIN WITHIN A RICE PANICLE (Chang *et al*., 1965)



A. ***RANJIT***



B. ***MAHSURI***



C. ***KAJOLI CHAKUA***

FIG. 3.1.1. GERMINATION TEST OF RICE SEEDS

FIG.3.1.2. SEEDLINGS READY FOR TRANSPLANTING

A. APPLICATION OF DISTILLED WATER (CONTROL)

B. APPLICATION OF $FeSO_4.7H_2O$ SOLUTION (TREATED)

FIG. 3.1.3. APPLICATION OF DISTILLED WATER TO THE CONTROL POTS AND $FeSO_4.7H_2O$ SOLUTION TO THE TREATED POTS

A. ***RANJIT***

B. ***MAHSURI***

C. ***KAJOLI CHAKUA***

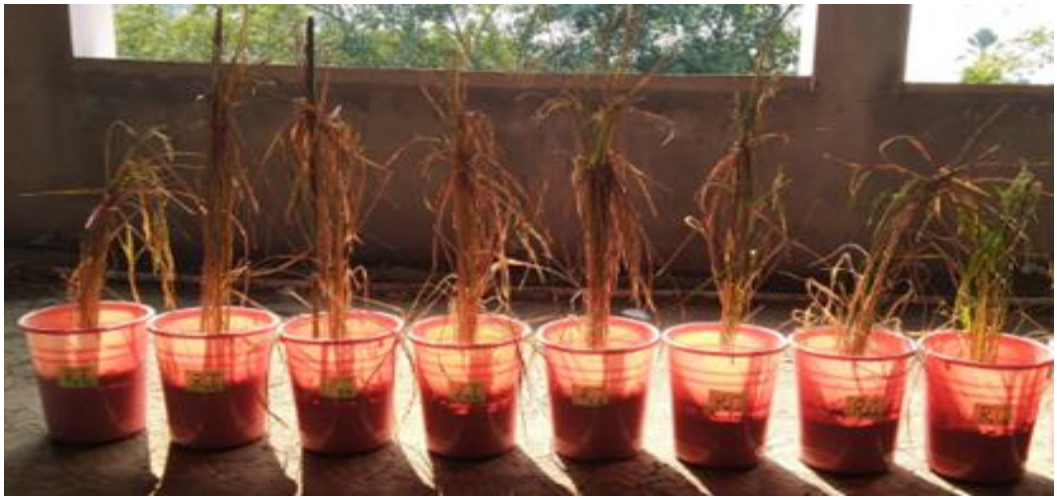
FIG. 3.1.4. RICE PLANTS AT MAXIMUM TILLERING STGAE

A. ***RANJIT***

B. ***MAHSURI***

C. ***KAJOLI CHAKUA***

FIG. 3.1.5. RICE PLANTS AT GRAIN FILLING STAGE



A. ***RANJIT***

B. ***MAHSURI***

C. ***KAJOLI CHAKUA***

FIG. 3.1.6. RICE PLANTS AT HARVESTING STAGE

a. GRAIN FILLING STAGE b. HARVESTING STAGE

A. *RANJIT*

a. GRAIN FILLING STAGE b. HARVESTING STAGE

B. *MAHSURI*

a. GRAIN FILLING STAGE b. HARVESTING STAGE

C. *KAJOLI CHAKUA*

FIG. 3.1.7. BROWN RICE, AFTER DEHUSKING AT GRAIN FILLING AND HARVESTING STAGE

3.2. Chemicals used

Acetic acid, acetone, ammonium acetate, ammonium chloride, ammonium hydroxide, calcium chloride, copper sulphate, diphenylamine indicator, di-potassium hydrogen phosphate, Di-ethylene Tri-amine Penta acetic Acid (DTPA), Ethylene Di-amine Tetra acetic Acid (EDTA), ethanol, ferric chloride, ferric nitrate, ferrous ammonium sulphate, ferrous sulphate, hydrochloric acid, hydroxylamine hydrochloride, hydrogen peroxide, magnesium oxide, methanol, methyl red indicator, nitric acid, o-dianisidine, orthophenanthroline, orthophosphoric acid, poly vinyl pyrrolidone (PVP), potassium dihydrogen phosphate, potassium dichromate, potassium persulfate, potassium thiocyanate, pyrogallol, sodium acetate, sodium carbonate, sodium fluoride, sodium potassium tartarate, sodium sulphate, sulfuric acid, Tri-ethanolamine (TEA) and tris-HCl were procured from MERCK, Bangalore; Bovine serum albumin, Folin-ciocalteu reagent, sodium hydroxide and tri-chloro acetic acid were procured from Sisco Research Laboratories Private Limited, Mumbai. All the chemicals were of analytical grade.

3.3. Analytical Methods

3.3.1. Analysis of plant sample

3.3.1.1. Determination of moisture content

Moisture content was determined by following the method of AOAC (1970).

For calculating the moisture content, 10 g of freshly collected sample was accurately weighed in aluminium moisture boxes and dried in an oven at 100℃ (±2℃) for 16 hours, then cooled in a dessicator and reweighed. This was repeated till a constant weight was recorded. The estimation was done in triplicate and the mean of all the three estimation was calculated.

Calculation :

$$\text{Moisture content (\% fresh weight basis)} = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Weight of the sample (g)}} \text{X}100$$

3.3.1.2. Determination of chlorophyll content

Chlorophyll content was determined according to the method of Arnon (1949).

1 g of fresh leaf sample was grinded with the addition of 20 ml 80% acetone, centrifuged at 5000 rpm for 5 min and then transferred the supernatant to a 100 ml volumetric flask. Then the residue was grinded with 20 ml of 80% acetone, centrifuged and transferred the supernatant to the volumetric flask. This procedure was repeated until the residue became colourless. Then the volume was made up to 100 ml with 80% acetone. The optical density of the solution was measured at 663 nm and 645 nm. Measurement was done against the reagent blank (80% acetone). The amount of chlorophyll was calculated using the formula:

mg total chlorophyll/g tissue = $[20.2(A_{645}) + 8.02(A_{663})] \times V/(1000 \times W)$

where, A = Absorbance at specific wavelength

V = Final volume of chlorophyll extract in 80 % acetone

W = Fresh weight of sample (g)

3.3.1.3. Determination of ash content

The ash content was estimated according to the method as described by A.O.A.C (1970).

5 g fine powdered moisture free sample was weighed in a pre weighed silica crucible, charred in low Bunsen flame and finally ignited at 600˚±25℃ for 6 hrs in the muffle furnace. After cooling, the weight of the crucible and ash was recorded.

Calculation

$$\text{Ash content (g/100g sample)} = \frac{\text{Weight of the ash (g)}}{\text{Weight of the sample (g)}} \text{ X } 100$$

The estimation was done in triplicate and the mean was recorded as percentage of ash content on dry weight basis.

Mineral composition

Preparation of mineral solution

The mineral solution was prepared according to the method described by AOAC (1970).

HCl and distilled water (1:1) mixture was added to the crucible containing ash. The crucible was kept on water bath at 100℃ and the solution was evaporated to dryness.

HCl and distilled water (2:1) mixture was added and the acid soluble portion obtained after evaporation was retained with filtration. The volume was made upto 100 ml with distilled water. The solution was used for the estimation of iron.

3.3.1.4. Determination of iron content

Iron content was determined colorimetrically according to the method of Wong (1928) using UV-VIS spectrophotometer.

To 6.5 ml of aliquot, 1 ml 30% sulphuric acid (H_2S0_4), 1 ml 7% potassium persulfate ($K_2S_2O_8$) solution and 1 ml of 40% potassium thiocyanate (KSCN) solution were added, respectively and mixed thoroughly. After 20 min, the optical density of the solution was measured at 540 nm against the reagent blank. The iron concentration was calculated out from the standard (Ferrous ammonium sulphate) curve which was prepared by using the iron solution of known strength in the range 10 μg to 50 μg.

The estimation was done in triplicate and their mean was recorded as mg of iron content per 100 g on dry weight basis.

3.3.1.5. Determination of peroxidase activity (EC 1.11.1.7)

The enzyme peroxidase was determined according to the method of Summer and Gjessing, 1943.

The enzyme was extracted by homogenizing 0.25 g fresh sample in ice cold 2.5 ml of phosphate buffer (0.1 M, pH 6.0). The material was strained through two folds of muslin cloth and centrifuged the homogenate at 16,000 rpm for 20 mins at 4℃. The supernatant was used for crude enzyme activity estimation.

The enzyme activity was assayed by mixing 1 ml of o-dianisidine, 0.5 ml of H_2O_2, 1 ml of phosphate buffer, 2.4 ml of distilled water and incubated at 25℃. The reaction mixture without H_2O_2 was used as blank. The reaction was started by adding 0.1 ml of the enzyme extract and stopped after 5 min by adding 1 ml of 2 N H_2SO_4. The optical density of the solution was measured at 430 nm. The measurement was done against a reagent blank.

One unit of total peroxidase activity has been defined as an increase in optical density by 1.0 under standard conditions (25℃, pH 6.0).

The total and specific activity of the enzyme was expressed as units/min/g fresh weight of the sample and units/mg protein, respectively.

3.3.1.6. Determination of superoxide dismutase activity (EC 1.15.1.1)

The enzyme superoxide dismutase was determined according to the method of Marklund and Marklund, (1974).

The enzyme was extracted from 0.25 g fresh sample with 2.5 ml 0.1 M sodium phosphate buffer (pH 7.5) containing 1% PVP. The extract was centrifuged at 10,000 rpm for 10 mins. The supernatant was used for crude enzyme activity estimation.

To a cuvette, 1.5 ml of 0.1 M Tris-HCl buffer (pH 8.2), 0.5 ml of 6 mM EDTA, 1 ml of 0.6 mM pyrogallol solution and 0.1 ml of enzyme extract were added. Absorbance was recorded at 420 nm after an interval of 30s up to 2 min. The reaction mixture without pyrogallol was taken as blank.

One unit of total SOD activity has been defined as change in ΔA/min/g fresh weight. ΔA denotes the difference in absorbance between control and sample.

The total and specific activity of the enzyme was expressed as units/min/g fresh weight of the sample and units/mg protein, respectively.

3.3.1.7. Determination of catalase activity (EC 1.11.1.6)

The enzyme catalase was determined according to the method of Beers *et al*., (1952).

The enzyme was extracted from 0.1 g fresh sample with 2.5 ml 0.1 M potassium phosphate buffer (pH 7.5). The extract was centrifuged at 10,000 rpm for 10 mins. The supernatant was used for crude enzyme activity estimation.

The enzyme estimation was done by transferring 2.90 ml of hydrogen peroxide solution [0.036 % (w/w)] into a quartz cuvette. Then the cuvette was placed in the spectrophotometer and allowed the substrate to equilibriate to 25℃. Then 0.10 ml of catalase extract was added to the cuvette. Then the solution was mixed immediately by inversion and the decrease in absorbance (240 nm**)** was monitored by taking one reading per second for 180 seconds. Then the time required for the A_{240} to decrease from 0.45 to 0.40 absorbance units was recorded. The measurement was done against a reagent blank.

One unit of total activity of catalase decomposes 1.0 μmol of H_2O_2 per minute at pH 7.0 at 25℃, while the H_2O_2 concentration falls from 10.3 mM to 9.2 mM. The rate of disappearance of H_2O_2 was followed by observing the rate of decrease in the absorbance at 240 nm.

The total and specific activity of the enzyme was expressed as units/min/g fresh weight of the sample and units/mg protein, respectively.

3.3.1.8. Determination of total soluble protein

Total soluble protein content of the three enzyme extracts were determined according to the method of Lowry *et al*., 1951.

To 0.1 ml of the respective enzyme extract, 0.9 ml distilled water was added to make up the volume. Then 5 ml of alkaline copper sulphate ($CuSO_4$) reagent was added, mixed well and incubated at room temperature for 10 mins. Then 0.5 ml of Folin-Ciocalteu reagent was added, mixed well and incubated at room temperature in dark for 30 mins. The optical density of the solution was measured at 660 nm against a reagent blank. The protein concentration was calculated out from the standard curve which was prepared by using the protein (Bovine Serum Albumin) solution of known strength (25 μg to 250 μg).

The estimation was done in triplicate and their mean was recorded as mg of protein content per ml of enzyme extract. The value was later converted to mg soluble protein present in respective enzyme extract prepared from 1 g fresh weight of the sample.

3.3.1.9. Determination of phytic acid content

Phytic acid content was determined according to the method of Wheeler and Ferrel (1971).

2 g dried sample was extracted in 50 ml 3% Trichloroacetic acid (TCA) for 30 min with mechanical shaker. Then the suspension was centrifuged and a 10 ml aliquot of the supernatant was taken and mixed with 4 ml of ferric chloride ($FeCl_3$) solution. After that the mixture was heated on boiling water bath for 45 min and centrifuged for 10-15 min and decanted the supernatant. The remaining precipitate was washed twice by 20-25 ml 3% TCA and heated on boiling water for 5-10 min and centrifuged. The process was repeated with water. Then the precipitate

was mixed with few ml (2 ml) of water and 3 ml of 1.5 N sodium hydroxide (NaOH). The mixture was again heated on boiling water bath for 30 mins. Then the volume was made up to 30 ml with water. The mixture was filtered through Whatman No. 2 filter paper and washed the precipitate with 60-70 ml hot water and the filtrate was discarded. The precipitate was dissolved with 40 ml hot 3.2 N nitric acid (HNO_3) into a volumetric flask. Finally, 15 ml aliquot was taken to another 100 ml volumetric flask and diluted up to 70 ml. 20 ml potassium thiocyanate (KSCN) was added and the reading was taken immediately (within 1 min) at 480 nm. A reagent blank was run with each set of sample**.** The phytic acid P concentration was calculated out from the standard (ferric nitrate, $Fe(NO_3)_3$) curve which was prepared by using the ferric nitrate solution of known strength (30 μg to 750 μg).

The following formula was used to calculate the phytic acid content of the sample.

Calculation :

$$\text{Phytate P (mg/100g sample)} = \frac{\mu g \text{ Fe X } 15}{\text{Weight of the sample (g)}}$$

3.3.2. Analysis of soil sample

Soil analysis was done before planting and after harvesting of the crop as mentioned above.

3.3.2.1. Determination of soil pH

The soil pH was determined with a glass electrode pH meter, in a 1:2 soil water suspension as described by Jackson (1973). 1:2 soil water suspension was prepared by taking 20g of soil and 40 ml of distilled water in 100 ml beaker. Then the suspension was shaken at regular intervals for half an hour. The pH meter was set at room temperature and calibrated by immersing the electrodes in different buffer solutions of pH 4.0, 7.0 and 9.2. Then the electrode was dipped into the soil water suspension and the reading of the pH was noted.

3.3.2.2. Determination of soil organic carbon

Rapid titration procedure of Walkley and Black (1934) was followed for the determination of organic carbon (OC) content of the soil.

Accurately weighed 1 g soil sample was taken in a 500 ml conical flask. 10 ml of 1 N $K_2Cr_2O_7$ (potassium dichromate) solution was added with the

help of a burette and mixed well with the soil. Then 20 ml concentrated H_2SO_4 (sulphuric acid) was added and swirled gently and vigorously for 1 min. The heat of dilution of H_2SO_4 raised the temperature to a maximum of 120℃. It was allowed to stand for 30 mins for chemical reactions. About 200 ml distilled water, 10 ml orthophosphoric acid, 10 ml sodium fluoride solution and 2-3 drops of diphenylamine indicator were added and was shaken well. Then the solution was titrated with 0.5 N ferrous ammonium sulphate solution till the colour changed from violet through blue to bright green. At the end point (bright green colour), the amount of ferrous ammonium sulphate solution used in titration was noted. A blank (without soil) was carried out in a similar manner.

Calculation :

Weight of the soil sample	= W g
Vol. of 0.5 N ferrous ammonium sulphate solution used in blank titration	= x ml
Vol. of 0.5 N ferrous ammonium sulphate solution used in back titration after oxidation of carbon	= y ml
Vol. of 0.5 N ferrous ammonium sulphate equivalent used for oxidation of carbon	= (x-y) ml
1 ml of 1 N ferrous ammonium sulphate	= 1 ml of $K_2Cr_2O_7$
x-y ml 0.5 N ferrous ammonium sulphate	= 0.5 (x-y) ml of $K_2Cr_2O_7$
Again, 1 ml of 1 N $K_2Cr_2O_7$	= 0.003g of organic carbon
0.5 (x-y) ml of 1 N $K_2Cr_2O_7$	= 0.5 (x-y) X 0.003 g organic carbon
W g soil contains 0.5 (x-y) X 0.003g organic carbon	
% Organic carbon in the soil	= 0.5 (x-y) X 0.003 X 100/W

The percent recovery of organic carbon by Walkley and Black method varies from soil to soil. In some soil, the recovery is about 77%, while in others, it may go high. So, the estimated value should be corrected by a recovery factor (f) which is 1.3 (i.e,100/77) for 77% recovery.

% Organic carbon in the soil = 0.5 (x-y) X 0.003 X 100 X f/W

3.3.2.3. Determination of Cation Exchange Capacity (CEC)

Cation exchange capacity (CEC) of soil was determined following neutral normal ammonium acetate leaching method (Jackson, 1973).

10 g of soil sample was weighed in a 500 ml conical flask. 50 ml of ammonium acetate solution was added to the soil samples, mixed thoroughly and left overnight. Then the contents of the beaker was transferred to a moist filter paper, seated inside a Buchner funnel under suction. The soil was continued to leach with ammonium acetate solution by pouring 25 ml at a time using in all 150 ml. The filtrate was kept for the determination of total and individual exchangeable bases. To the soil on the filter paper, a pinch of ammonium chloride (NH_4Cl) crystal was added and leached with 30 ml ethanol. The residue was continued to wash till the filtrate was free of chloride. Then the residue and filter paper was transferred to a 500 ml distillation flask. Then 0.5 g of magnesium oxide (MgO) powder and 200-300 ml distilled water were added. Then the flask was connected to the distillation apparatus immediately after addition of MgO powder. A 250 ml beaker was placed, containing 25 ml of 0.1 N H_2SO_4 under the condenser of the distillation apparatus to receive ammonia (NH_3). Distillation was started by heating the distillation apparatus. The heating was continued till no ammonia was evolved through the end of the condenser. It was confirmed by turning of red litmus paper to blue. The excess acid was back titrated with 0.1 N sodium hydroxide (NaOH) solution. One reagent blank was run with the same volume of ammonium acetate.

Observations :

Weight of the soil sample taken = 10 g

Vol. of 0.1 N NaOH required for sample titration = X ml

Vol. of 0.1 N NaOH required for blank titration = Y ml

Calculations :

meq. of NaOH used for sample titration = X x 0.1 = a

meq. of NaOH used for blank titration = Y x 0.1 = b

meq. of H_2SO_4 consumed for NH_3 absorption = (b-a) x 0.1 = c

Now, 10 g of soil contains = c meq.

Hence 100 g of soil contains = c/10 x 100 meq.

3.3.2.4. Determination of available iron in the growth medium

Available iron in soil was determined colorimetrically by orthophenanthroline method (Krishnamurti *et al*., 1970).

The extracting reagent was prepared by taking 1.967 g of Di-ethylene Tri-amine Penta acetic Acid (DTPA), 1.470 g Calcium chloride ($CaCl_2.2H_2O$) and 13.3 ml of Tri-ethanolamine (TEA), in 100 ml beaker with doubled distilled water and diluted approximately to 900 ml. The pH of the solution was adjusted with pH meter to 7.3 by adding dilute hydrochloric acid (1:1). While stirring, the volume of the extracting solution was made to 1 L.

10 g of soil was weighed and transferred to a 150 ml volumetric flask and 20 ml DTPA extracting solution was added to it. The content of the volumetric flask was shaken on electric shaker for 120 mins. Then the content was filtered through Whatman No. 42 filter paper. A blank was prepared by adding all the solutions except soil by shaking and filtration.

Solutions of different concentrations of iron were prepared by adding 0.0, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 ml of $5x10^{-4}$ M ferrous ammonium sulphate solution into fourteen separate 5 ml volumetric flasks. To each flask, 2.0 ml to 0.0 ml of $5x10^{-4}$ M 1,10- phenanthroline solution was added

in such a way that the total volume of ferrous ammonium sulphate and 1,10-phenanthroline solutions remained 2.0 ml. The solutions were made upto the mark after adding 1.0 ml of acetate acetic acid buffer and 0.5 ml of 1.0 % hydroxylamine hydrochloride solution to each flask. The absorbance readings were taken against the reagent blank for each solution at 512 nm after 15 mins of mixing the reagents for uniform concentrations.

3.3.4. Statistical analysis

The mean data of three varieties were analysed statistically by using paired t-test in MS-Excel for comparison between control and treated, at 5% significant level.

CHAPTER IV

EXPERIMENTAL FINDINGS

The experimental findings of the present study **"Studies on metabolism of iron in rice"** are presented in this chapter.

4.1. Moisture content

4.1.1. Moisture content of rice leaves

The moisture content of rice leaves at three different growth stages in control and treated plants are presented in Table 4.1.1 and FIG. 4.1.1. It was observed that the variation in moisture content of rice leaves between the control and treated plants were non significant.

At maximum tillering stage, the highest moisture content in control (75.50±0.20%) and in treated (82.36±0.15%) were observed in *Kajoli chakua* and the lowest in control (65.06±0.13%) and in treated (67.55±0.38%) were observed in *Mahsuri*. The average moisture content was found to be 69.89±5.26% in control and 73.36±7.90% in treated plants.

At grain filling stage, the highest moisture content in control (72.97±0.02%) and in treated (73.16±0.03%) were observed in *Kajoli chakua* and the lowest in control (71.46±0.01%) and in treated (65.87±0.02%) were observed in *Mahsuri*. The average moisture content was found to be 72.45±0.86% in control and 70.62±4.11% in treated plants.

At harvesting stage, the highest moisture content in control (9.83±0.02%) and in treated (10.01±0.10%) were observed in *Mahsuri* and *Ranjit*, respectively and the lowest in control (7.26±0.07%) and in treated (7.15±0.01%) were observed in *Kajoli chakua* and *Mahsuri*, respectively. The average moisture content was found to be 8.62±1.29% in control and 8.66±1.43% in treated plants.

4.1.2. Moisture content of paddy and brown rice

Paddy

The moisture content of paddy at two different growth stages in control and treated plants are presented in Table 4.1.2 and FIG. 4.1.2(a). It was observed that the variation in moisture content of paddy between the control and treated plants were non significant.

At grain filling stage, the highest moisture content in control (13.76±0.40%) and in treated (13.60±0.20%) were observed in *Ranjit* and the lowest in control (12.93±0.11%) and in treated (11.33±0.30%) were observed in *Mahsuri*. The average moisture content was found to be 13.40±0.42% in control and 12.73±1.22% in treated plants.

At harvesting stage, the highest moisture content in control (13.93±0.05%) and in treated (14.06±0.23%) were observed in *Mahsuri* and the lowest in control (12.73±0.23%) and in treated (12.06±0.11%) were observed in *Kajoli chakua*. The average moisture content was found to be 13.33±0.60% in control and 12.95±1.01% in treated plants.

Brown rice

The moisture content of brown rice at two different growth stages in control and treated plants are presented in Table 4.1.2 and FIG. 4.1.2(b). It was observed that the variation in moisture content of brown rice among the control and treated plants were non significant.

At grain filling stage, the highest moisture content in control (12.29±0.05%) and in treated (11.98±0.06%) were observed in *Kajoli chakua* and the lowest in control (11.24±0.04%) was observed in *Ranjit* and in treated (11.28±0.04%) was observed in *Mahsuri*. The average moisture content was found to be 11.71±0.53% in control and 11.54±0.38% in treated plants.

At harvesting stage, the highest moisture content in control (11.61±0.04%) and in treated (11.44±0.04%) were observed in *Kajoli chakua.* The lowest moisture content in control (10.11±0.06%) and in treated (10.43±0.09%) were observed in *Ranjit.* The average moisture content was found to be 10.87±0.75% in control and 10.86±0.52% in treated plants.

4.2. Total Chlorophyll content

4.2.1. Total Chlorophyll content of rice leaves

The chlorophyll content (on fresh weight basis) of rice leaves at three different growth stages in control and treated plants are presented in Table 4.2.1 and FIG. 4.2.1. It was observed that the variation in chlorophyll content of rice leaves between the control and treated plants were significant.

At maximum tillering stage, the highest chlorophyll content in control (1.28±0.02 mg/g) and in treated (1.01±0.02 mg/g) were observed in *Kajoli chakua* and the lowest in control (1.17±0.02 mg/g) and in treated (0.88±0.02 mg/g) were observed in *Mahsuri*. The average chlorophyll content was found to be 1.23±0.05 mg/g in control and 0.94±0.06 mg/g in treated plants.

At grain filling stage, the highest chlorophyll content in control (2.33±0.03 mg/g) and in treated (1.9±0.02 mg/g) were observed in *Kajoli chakua* and the lowest in control (2.07±0.02 mg/g) and in treated (1.73±0.03 mg/g) were observed in *Mahsuri*. The average chlorophyll content was found to be 2.2±0.13 mg/g in control and 1.81±0.10 mg/g in treated plants.

At harvesting stage, the highest chlorophyll content in control (0.57±0.01 mg/g) and in treated (0.49±0.04 mg/g) were observed in *Kajoli chakua* and the lowest in control (0.38±0.01 mg/g) and in treated (0.29±0.02 mg/g) were observed in *Mahsuri*. The average chlorophyll content was found to be 0.46±0.09 mg/g in control and 0.36±0.10 mg/g in treated plants.

4.3. Ash content

4.3.1. Ash content of rice leaves

The ash content of rice leaves at three different growth stages in control and treated plants are presented in Table 4.3.1 and FIG. 4.3.1. It was observed that the variation in ash content of rice leaves between the control and treated plants were non significant.

At maximum tillering stage, the highest ash content in control (14.50±0.55%) and in treated (15.46±0.61%) were observed in *Kajoli chakua* and the lowest in control (12.00±0.91%) and in treated (14.84±0.96%) were observed in

Ranjit. The average ash content was found to be 13.66±1.43 % in control and 15.11±0.31% in treated plants.

At grain filling stage, the highest ash content in control (16.10±0.12%) and in treated (14.72±0.08%) were observed in *Kajoli chakua* and *Ranjit* , respectively and the lowest in control (14.25±1.65%) and in treated (12.29±0.12%) were observed in *Ranjit* and *Kajoli chakua,* respectively. The average ash content was found to be 14.93±1.01% in control and 13.15±1.35% in treated plants.

At harvesting stage, the highest ash content in control (20.93±0.12 %) and in treated (19.73±0.46%) were observed in *Ranjit* and *Mahsuri,* respectively and the lowest in control (17.81±0.20%) and in treated (16.08±0.08%) were observed in *Kajoli chakua* and *Ranjit,* respectively. The average ash content was found to be 19.10±1.62% in control and 17.71±1.85% in treated plants.

4.3.2. Ash content of brown rice

The ash content of brown rice at grain filling stage in control and treated plants are presented in Table 4.3.2(a) and Fig. 4.3.2(a). It was observed that the variation in ash content of brown rice between the control and treated plants were non significant.

The highest ash content in control (2.33±0.11%) and in treated (1.87±0.12%) were observed in *Mahsuri* and the lowest in control (1.70±0.02%) and in treated (1.53±0.11%) was observed in *Ranjit* and *Kajoli chakua,* respectively. The average ash content was found to be 1.97±0.32% in control and 1.65±0.19% in treated plants.

Positional variation in ash content of brown rice at harvesting stage in control and treated plants are presented in Table 4.3.2(b) and FIG. 4.3.2(b). It was observed that the ash content of brown rice only in top primary rachis varied significantly between the control and treated plants.

In top primary rachis, the highest ash content in both control (1.94±0.04%) and in treated (1.82±0.02%) were observed in *Ranjit.* However, the lowest ash content in both control (1.76±0.05%) and in treated (1.63±0.15%) were observed in *Kajoli chakua.* The average ash content was found to be 1.83±0.09% in control and 1.72±0.09% in treated plants.

In top secondary rachis, the highest ash content in control (2.00±0.40%) and in treated (1.84±0.04%) were observed in *Ranjit* and *Mahsuri,* respectively and the lowest ash content in control (1.70±0.10%) and in treated (1.63±0.23 %) were observed in *Kajoli chakua.* The average ash content was found to be 1.89±0.16% in control and 1.74±0.10% in treated plants.

In middle primary rachis, the highest ash content in control (1.97±0.02%) and in treated (1.82±0.02 %) were observed in *Ranjit* and the lowest in control (1.61±0.02%) and in treated (1.52±0.28%) were observed in *Mahsuri.* The average ash content was found to be 1.82±0.19% in control and 1.70±0.16% in treated plants.

In middle secondary rachis, the highest ash content in control (2.09±0.10%) and in treated (1.78±0.06%) were observed in *Mahsuri* and *Ranjit,* respectively and the lowest in control (1.83±0.05%) and in treated (1.60±0.34 %) were observed in *Kajoli chakua.* The average ash content was found to be 1.93±0.14% in control and 1.68±0.09% in treated plants.

In bottom primary rachis, the highest ash content in control (2.40±0.40%) and in treated (1.86±0.05%) were observed in *Ranjit* and *Kajoli chakua,* respectively and the lowest in control (1.90±0.10%) and in treated (1.68±0.04%) were observed in *Kajoli chakua* and *Mahsuri,* respectively. The average ash content was found to be 2.07±0.28% in control and 1.78±0.09% in treated plants.

In bottom secondary rachis, the highest ash content in control (1.82±0.04%) and in treated (1.96±0.04%) were observed in *Ranjit* and the lowest in control (1.60±0.06 %) and in treated (1.61±0.02%) were observed in *Mahsuri.* The average ash content was found to be 1.75±0.09% in control and 1.76±0.17% in treated plants.

4.3.3. Ash content of rice husk

The ash content of rice husk at two different growth stages in control and treated plants are presented in Table 4.3.4 and Fig. 4.3.4. It was observed that the ash content of rice husk at grain filling stage varied significantly between the control and treated plants.

At grain filling stage, both the highest ash content in control (14.52±0.04%) and in treated (13.42±0.04%) were observed in *Ranjit* and the lowest in control (12.53±0.22%) and in treated (10.28±0.06%) were observed in *Mahsuri*. The average ash content was found to be 13.51±0.99% in control and 11.60±1.62% in treated plants.

At harvesting stage, the highest ash content in control (14.11±0.15%) and in treated (12.52±0.12%) were observed in *Ranjit* and *Kajoli chakua,* respectively and the lowest in control (12.47±0.16 %) and in treated (11.39±0.22 %) were observed in *Mahsuri*. The average ash content was found to be 13.26±0.82% in control and 11.99±0.56% in treated plants.

4.4. Iron content

4.4.1. Iron content of rice leaves

The iron content of rice leaves at three different growth stages in control and treated plants are presented in Table 4.4.1 and FIG. 4.4.1. It was observed that the iron content of rice leaves at grain filling stage varied significantly between the control and treated plants.

At maximum tillering stage, the highest iron content in control (27.64±0.06 mg/100g) and in treated (54.17±0.25 mg/100g) were observed in *Ranjit* and the lowest in control (16.12±0.20 mg/100g) and in treated (33.91±0.03 mg/100g) were observed in *Mahsuri*. The average iron content was found to be 23.68±6.55 mg/100g in control and 41.36±11.14 mg/100g) in treated plants.

At grain filling stage, the highest iron content in control (58.81±0.26 mg/100g) and in treated (75.01±0.26 mg/100g) were observed in *Ranjit* and the lowest in control (18.39±0.12 mg/100g) and in treated (36.76±0.12 mg/100g) were observed in *Mahsuri*. The average iron content was found to be 41.42±20.79 mg/100g in control and 58.01±19.47 mg/100g in treated plants.

At harvesting stage, the highest iron content in control (57.58±0.01 mg/100g) and in treated (69.71±0.19 mg/100g) were observed in *Ranjit* and the lowest in control (24.77±0.21 mg/100g) and in treated (26.75±0.14 mg/100g) were observed in *Mahsuri.* The average iron content was found to be 39.16±16.77 mg/100g in control and 45.36±22.04 mg/100g in treated plants.

4.4.2. Iron content of brown rice

The iron content of brown rice at grain filling stage in control and treated plants are presented in Table 4.4.2(a) and FIG. 4.4.2(a). It was observed that the iron content of brown rice at grain filling stage varied significantly between the control and treated plants.

The highest iron content in control (2.18±0.08 mg/100g) and in treated (5.92±0.10 mg/100g) were observed in *Ranjit* and the lowest in control (1.17±0.04 mg/100g) and in treated (4.18±0.03 mg/100g) were observed in *Mahsuri*. The average iron content was found to be 1.74±5.17 mg/100g in control and 5.08±8.70 mg/100g in treated plants.

Positional variation in iron content of brown rice at harvesting stage in control and treated plants are presented in Table 4.4.2(b) and FIG. 4.4.2(b). It was observed that the iron content of brown rice in all the grain positions at harvesting stage varied significantly among the control and treated plants.

In top primary rachis, the highest iron content in control (5.18±0.15 mg/100g) and in treated (11.09±0.22 mg/100g) were observed in *Ranjit* and the lowest in control (3.26±0.09 mg/100g) and in treated (7.94±0.01 mg/100g) were observed in *Mahsuri.* The average iron content was found to be 4.10±0.98 mg/100g in control and 9.13±1.70 mg/100g in treated plants.

In top secondary rachis, the highest iron content in control (4.85±0.08 mg/100g) and in treated (10.62±0.13 mg/100g) were observed in *Ranjit* and the lowest in control (3.04±0.05 mg/100g) and in treated (6.89±0.07 mg/100g) were observed in *Mahsuri.* The average iron content was found to be 3.83±0.92 mg/100g in control and (8.61±1.87 mg/100g) in treated plants.

In middle primary rachis, the highest iron content in control (4.77±0.15 mg/100g) and in treated (10.54±0.26 mg/100g) were observed in *Ranjit* and the lowest in control (2.71±0.04 mg/100g) and in treated (6.77±0.11 mg/100g) were observed in *Mahsuri.* The average iron content was found to be 3.67±1.03 mg/100g in control and 8.44±1.92 mg/100g in treated plants.

In middle secondary rachis, the highest iron content in control (4.04 mg/100g) and in treated (10.26 mg/100g) were observed in *Ranjit* and the lowest in control (2.28±0.11 mg/100g) and in treated (6.73±0.06 mg/100g) were observed in

Mahsuri. The average iron content was found to be 3.23±0.89 mg/100g in control and 8.28±1.80 mg/100g in treated plants.

In bottom primary rachis, the highest iron content in control (3.91±0.11 mg/100g) and in treated (10.21±0.32 mg/100g) were observed in *Ranjit* and the lowest in control (1.97±0.13 mg/100g) and in treated (6.01±0.07 mg/100g) were observed in *Mahsuri.* The average iron content was found to be 3.04±0.98 mg/100g in control and 7.96±2.11 mg/100g in treated plants.

In bottom secondary rachis, the highest iron content in control (3.76±0.11 mg/100g) and in treated (9.67±0.17 mg/100g) were observed in *Ranjit* and the lowest in control (1.86±0.05 mg/100g) and in treated (5.88±0.06 mg/100g) were observed in *Mahsuri*. The average iron content was found to be 2.94±0.98 mg/100g in control and 7.68±1.89 mg/100g in treated plants.

4.4.3. Iron content of rice husk

The iron content of rice husk at two different growth stages in control and treated plants are presented in Table 4.4.3 and FIG. 4.4.3. It was observed that the iron content of rice husk varied significantly both, at grain filling and harvesting stage between the control and treated plants.

At grain filling stage, the highest iron content in control (5.50±0.30 mg/100g) and in treated (9.37±0.01 mg/100g) were observed in *Ranjit* and the lowest in control (3.11±0.02 mg/100g) and in treated (6.96±0.10 mg/100g) were observed in *Mahsuri.* The average iron content was found to be 4.09±1.24 mg/100g in control and 8.41±1.27 mg/100g in treated plants.

At harvesting stage, the highest iron content in control (8.91±0.01 mg/100g) and in treated (12.07±0.30 mg/100g) were observed in *Ranjit* and the lowest in control (6.95±0.18 mg/100g) and in treated (11.17±0.32 mg/100g) were observed in *Mahsuri.* The average iron content was found to be 7.94±0.98 mg/100g in control and 11.56±0.45 mg/100g in treated plants.

4.5. Peroxidase activity

4.5.1(a). Total activity of peroxidase extract prepared from rice leaves

The total activity of peroxidase extract prepared from rice leaves at three different growth stages in control and treated plants are presented in Table

4.5.1 and FIG. 4.5.1(a). In the present study, the total activity of peroxidase for three rice varieties was found to be significantly higher in treated than in control only at maximum tillering stage.

In almost all the three stages, the highest total activity of peroxidase was found in *Mahsuri* followed by *Kajoli chakua* and *Ranjit*, respectively.

At maximum tillering stage, the highest total activity in control (22.81±0.01 units/min/g fresh weight) and in treated (23.12±0.07 units/min/g fresh weight) were observed in *Mahsuri* and the lowest in control (21.14±0.04 units/min/g fresh weight) and in treated (21.86±0.11 units/min/g fresh weight) were observed in *Ranjit*. The average total activity was found to be 22.08±0.85 units/min/g fresh weight in control and 22.59±0.65 units/min/g fresh weight in treated plants.

At grain filling stage, the highest total activity in control (22.80±0.05 units/min/g fresh weight) was observed in *Kajoli chakua* and in treated (23.00±0.20 units/min/g fresh weight) was observed in *Mahsuri* and the lowest in control (21.74±0.03 units/min/g fresh weight) and in treated (21.96±0.02 units/min/g fresh weight) were observed in *Ranjit*. The average total activity was found to be 22.33±0.54 units/min/g fresh weight in control and 22.39±0.54 units/min/g fresh weight in treated plants.

At harvesting stage, the highest total activity in control (24.02±0.03 units/min/g fresh weight) was observed in *Mahsuri* and in treated (24.14±0.04 units/min/g fresh weight) were observed in *Kajoli chakua* and the lowest (22.64±0.04 units/min/g fresh weight) in control was observed in *Ranjit* and (22.82±0.03 units/min/g fresh weight) in treated was observed in *Mahsuri.* The average total activity was found to be 23.42±0.71 units/min/g fresh weight in control and 23.42±0.66 units/min/g fresh weight in treated plants.

4.5.1(b). Specific activity of peroxidase extract prepared from rice leaves

The specific activity of peroxidase extract prepared from rice leaves at three different growth stages in control and treated plants are presented in Table 4.5.1 and FIG. 4.5.1(b). The specific activity of peroxidase extract prepared from leaves for three rice varieties was found to be non significant at all the stages.

At maximum tillering stage, the highest specific activity in control (50.34±1.10 units/mg protein) and in treated (78.09±2.63 units/mg protein) were

observed in *Ranjit* and the lowest in control (48.47±1.36 units/mg protein) was observed in *Kajoli chakua* and in treated (57.80±1.36 units/mg protein) was observed in *Mahsuri*. The average specific activity was found to be 49.46±0.94 units/mg protein in control and 67.01±10.27 units/mg protein in treated plants.

At grain filling stage, the highest specific activity in control (54.28±1.41 units/mg protein) was observed in *Kajoli chakua* and in treated (60.52±1.31 units/mg protein) were observed in *Mahsuri* and the lowest in control (49.92±2.20 units/mg protein) was observed in *Mahsuri* and in treated (55.55±1.37 units/mg protein) was observed in *Kajoli chakua.* The average specific activity was found to be 52.41±2.24 units/mg protein in control and 58.47±2.60 units/mg protein in treated plants.

At harvesting stage, the highest specific activity in control (42.15±1.13 units/mg protein) was observed in *Mahsuri* and in treated (55.49±1.19 units/mg protein) was observed in *Ranjit* and the lowest in control (36.33±1.24 units/mg protein) was observed in *Kajoli chakua* and in treated (43.88±1.10 units/mg protein) was observed in *Mahsuri.* The average specific activity was found to be 38.95±2.95 units/mg protein in control and 48.02±6.47 units/mg protein in treated plants.

4.5.2(a). Total activity of peroxidase extract prepared from brown rice

The total activity of peroxidase extract prepared from brown rice at two different growth stages in control and treated are presented in Table 4.5.2 and FIG. 4.5.2(a). At grain filling stage, the total activity of peroxidase of brown rice was significantly higher in treated plants than in control.

At grain filling stage, the highest total activity in control (20.06±0.44 units/min/g fresh weight) was observed in *Kajoli chakua* and in treated (20.86±0.22 units/min/g fresh weight) were observed in *Mahsuri* and the lowest in control (19.33±0.31 units/min/g fresh weight) and in treated (20.26±0.24 units/min/g fresh weight) were observed in *Ranjit.* The average total activity was found to be 19.81±0.41 units/min/g fresh weight in control and 20.53±0.30 units/min/g fresh weight in treated plants.

At harvesting stage, the highest total activity in control (21.79±0.33 units/min/g fresh weight) and in treated (22.10±0.14units/min/g fresh weight) were

observed in *Mahsuri* and the lowest in control (20.76±0.12 units/min/g fresh weight) was observed in *Ranjit* and in treated (21.65±0.61 units/min/g fresh weight) was observed in *Kajoli chakua*. The average total activity was found to be 21.19±0.53 units/min/g fresh weight in control and 21.50±0.35 units/min/g fresh weight in treated plants.

4.5.2 (b). Specific activity of peroxidase extract prepared from brown rice

The specific activity of peroxidase extract prepared from brown rice at two different growth stages in control and treated plants are presented in Table 4.5.2. and FIG. 4.5.2(b). It was significantly higher in treated plants at grain filling stage only.

At grain filling stage, the highest specific activity of peroxidase was found in *Ranjit* for treated and *Kajoli chakua* for control and for both, the least was detected in *Mahsuri*, respectively

At grain filling stage, the highest specific activity in control (2.99±0.01 units/mg protein) was observed in *Kajoli chakua* and in treated (3.61±0.05 units/mg protein) was observed in *Ranjit* and the lowest in control (2.50±0.01 units/mg protein) and in treated (2.89±0.03 units/mg protein) was observed in *Mahsuri.* The average specific activity was found to be 2.80±0.26 units/mg protein in control and 3.26±0.36 units/mg protein in treated plants.

At harvesting stage, the highest specific activity in control (2.21±0.02 units/mg protein) was observed in *Mahsuri* and in treated (3.84±0.04 units/mg protein) was observed in *Ranjit* and the lowest in control (1.90±0.01 units/mg protein) was observed in *Kajoli chakua* and in treated (2.03±0.02 units/mg protein) were observed in *Mahsuri*. The average specific activity was found to be 2.07±0.15 units/mg protein in control and 2.73±0.97 units/mg protein in treated plants.

4.6. Superoxide dismutase activity

4.6.1 (a). Total activity of superoxide dismutase extract prepared from rice leaves

The total activity of superoxide dismutase extract prepared from rice leaves at three different growth stages in control and treated plants are presented in Table 4.6.1 and FIG. 4.6.1(a).

The total activity of superoxide dismutase for three rice varieties was found to be significantly higher in treated than in control in all the three stages. The highest total activity of superoxide dismutase was found in *Ranjit* followed by *Mahsuri* and *Kajoli chakua* at maximum tillering stage. In the later two stages, the highest total activity of superoxide dismutase was found in *Ranjit* followed by *Kajoli chakua* and *Mahsuri*.

At maximum tillering stage, the highest total activity in control (0.75±0.05 units/min/g fresh weight) and in treated (1.25±0.01 units/min/g fresh weight) were observed in *Ranjit* and the lowest in control (0.50±0.01 units/min/g fresh weight) was observed in *Mahsuri* and *Kajoli chakua* and in treated (0.75±0.0 units/min/g fresh weight) was observed in *Kajoli chakua*. The average total activity was found to be 0.58±0.14 units/min/g fresh weight in control and 1.00±0.25 units/min/g fresh weight in treated plants.

At grain filling stage, the highest total activity in control (1.25±0.03 units/min/g fresh weight) was observed in *Ranjit* and in treated (1.50±0.01 units/min/g fresh weight) was observed in *Ranjit* and *Kajoli chakua* and the lowest in control (0.50±0.02 units/min/g fresh weight) and in treated (1.00±0.05 units/min/g fresh weight) were observed in *Mahsuri*. The average total activity was found to be 0.91±0.38 units/min/g fresh weight in control and 1.33±0.28 units/min/g fresh weight in treated plants.

At harvesting stage, the highest total activity in control (1.25±0.02 units/min/g fresh weight) and in treated (1.50±0.01 units/min/g fresh weight) were observed in *Ranjit* and the lowest in control (0.25±0.01 units/min/g fresh weight) and in treated (0.50±0.03 units/min/g fresh weight) were observed in *Mahsuri.* The average total activity was found to be 0.75±0.50 units/min/g fresh weight in control and 1.08±0.52 units/min/g fresh weight in treated plants.

4.6.1 (b). Specific activity of superoxide dismutase extract prepared from rice leaves

The specific activity of superoxide dismutase extract prepared from rice leaves at three different growth stages in control and treated plants are presented in Table 4.6.1 and FIG. 4.6.1(b).

In the present study, the specific activity of superoxide dismutase for three rice varieties was found to be significantly higher in treated than in control only at grain filling stage. At grain filling stage, the highest specific activity of superoxide dismutase was found to be the highest in *Ranjit* followed by *Kajoli chakua* and *Mahsuri* for treated, whereas in control, the same was found to be the highest for *Kajoli chakua* followed by *Ranjit* and *Mahsuri.*

At maximum tillering stage, the highest specific activity in control (2.34±0.22 units/mg protein) and in treated (2.60±0.31 units/mg protein) was observed in *Ranjit* and the lowest in control (1.13±0.20 units/mg protein) and in treated (1.59±0.22 units/mg protein) were observed in *Mahsuri*. The average specific activity was found to be 1.69±0.60 units/mg protein in control and 2.23±0.55 units/mg protein in treated plants.

At grain filling stage, the highest specific activity in control (3.12±0.20 units/mg protein) was observed in *Kajoli chakua* and in treated (4.05±0.32 units/mg protein) was observed in *Ranjit* and the lowest in control (1.85±0.21 units/mg protein) and in treated (2.63±0.30 units/mg protein) were observed in *Mahsuri*. The average specific activity was found to be 2.67±0.71 units/mg protein in control and 3.44±0.73 units/mg protein in treated plants.

At harvesting stage, the highest specific activity in control (2.45±0.21 units/mg protein) and in treated (4.05±0.32 units/mg protein) were observed in *Ranjit* and the lowest in control (0.55±0.22 units/mg protein) and in treated (1.04±0.21 units/mg protein) were observed in *Kajoli chakua.* The average specific activity was found to be 1.62±0.97 units/mg protein in control and 2.79±1.56 units/mg protein in treated plants.

4.6.2 (a). Total activity of superoxide dismutase extract prepared from brown rice

The total activity of superoxide dismutase extract prepared from brown rice at two different growth stages in control and treated plants are presented in Table 4.6.1. and FIG. 4.6.1(a). The variation in the total activity of SOD in brown rice was found to be non significant regarding different levels of soil iron content.

At grain filling stage, the highest total activity in control (1.50±0.02 units/min/g fresh weight) was observed in *Ranjit* and in treated (1.50±0.01

units/min/g fresh weight) were observed in *Kajoli chakua* and the lowest in control (0.50±0.01 units/min/g fresh weight) was observed in *Mahsuri* and in treated (1.00±0.05 units/min/g fresh weight) was observed in *Ranjit*. The average total activity was found to be 0.91±0.52 units/min/g fresh weight in control and 1.25±0.25 units/min/g fresh weight in treated plants.

At harvesting stage, the highest total activity in control (1.00±0.02 units/min/g fresh weight) was observed in *Kajoli chakua* and in treated (1.50±0.03 units/min/g fresh weight) were observed in *Ranjit* and *Mahsuri* and the lowest in control (0.25±0.03 units/min/g fresh weight) was observed in *Mahsuri* and in treated (1.25±0.02 units/min/g fresh weight) was observed in *Kajoli chakua.* The average total activity was found to be 0.66±0.38 units/min/g fresh weight in control and 1.41±0.14 units/min/g fresh weight in treated plants.

4.6.2 (b). Specific activity of superoxide dismutase extract prepared from brown rice

The specific activity of superoxide dismutase extract prepared from brown rice at two different growth stages in control and treated plants are presented in Table 4.6.2. and FIG. 4.6.2(b). At both the stages, the specific activity of SOD was found to be non significant irrespective of level of iron content of soil.

At grain filling stage, the highest specific activity in control (1.72±0.01 units/mg protein) was observed in *Ranjit* and in treated (1.97±0.02 units/mg protein) was observed in *Kajoli chakua* and the lowest in control (0.57±0.03 units/mg protein) and in treated (1.27±0.03 units/mg protein) were observed in *Mahsuri.* The average specific activity was found to be 1.05±0.59 units/mg protein in control and (1.72±0.39 units/mg protein) in treated plants.

At harvesting stage, the highest specific activity in control (0.98±0.02 units/mg protein) and in treated (2.20±0.03 units/mg protein) was observed in *Ranjit* and the lowest in control (0.25±0.01 units/mg protein) was observed in *Mahsuri* and in treated (1.28±0.02 units/mg protein) was observed in *Kajoli chakua*. The average specific activity was found to be 0.68±0.38 units/mg protein in control and 1.70±0.02 units/mg protein in treated plants.

4.7. Catalase activity

4.7.1 (a). Total activity of catalase extract prepared from rice leaves

The total activity of catalase extract prepared from rice leaves at three different growth stage in control and treated plants are presented in Table 4.7.1 and FIG. 4.7.1(a).

The total activity of catalase from leaves for three rice varieties was found to be significantly higher in treated than in control only at maximum tillering stage. At maximum tillering stage, the highest total activity of catalase was found in *Kajoli chakua* followed by *Ranjit* and *Mahsuri*.

At maximum tillering stage, the highest total activity in control (109.37±0.60 units/min/g fresh weight) and in treated (122.06±1.38 units/min/g fresh weight) were observed in *Kajoli chakua* and the lowest in control (79.50±0.69 units/min/g fresh weight) and in treated (92.62±0.32 units/min/g fresh weight) were observed in *Mahsuri*. The average total activity was found to be 92.56±15.28 units/min/g fresh weight in control and 107.10±14.72 units/min/g fresh weight in treated plants.

At grain filling stage, the highest total activity in control (107.87±0.52 units/min/ g fresh weight) and in treated (114.50±0.48 units/min/g fresh weight) were observed in *Ranjit* and the lowest in control (83.43±0.22 units/min/g fresh weight) and in treated (80.50±0.09 units/min/g fresh weight) were observed in *Kajoli chakua* and *Mahsuri*, respectively. The average total activity was found to be 91.95±13.79 units/min/g fresh weight in control and 97.50±17.00 units/min/ g fresh weight in treated plants.

At harvesting stage, the highest total activity in control (80.37±0.44 units/min/g fresh weight) and in treated (85.18±0.53units/min/g fresh weight) were observed in *Ranjit* and *Kajoli chakua*, respectively and the lowest in control (74.18±0.14 units/min/g fresh weight) and in treated (76.37±0.18 units/min/g fresh weight) was observed in *Mahsuri.* The average total activity was found to be 77.74±3.19 units/min/g fresh weight in control and 81.91±4.82 units/min/g fresh weight in treated plants.

4.7.1 (b). Specific activity of catalase extract prepared from rice leaves

The specific activity of catalase extract prepared from rice leaves at three different growth stages in control and treated plants are presented in Table 4.7.1 and FIG. 4.7.1(b).

In the present study, the specific activity of catalase for three rice varieties was found to be significantly higher in treated than in control only at maximum tillering stage. At maximum tillering stage, the highest specific activity of catalase was found in *Ranjit* followed by *Kajoli chakua* and *Mahsuri*.

At maximum tillering stage, the highest specific activity in control (71.62±0.32 units/mg protein) and in treated (80.16±0.40 units/mg protein) were observed in *Ranjit* and the lowest in control (53.71±0.29 units/mg protein) and in treated (59.75±0.26 units/mg protein) were observed in *Mahsuri*. The average specific activity was found to be 64.70±9.62 units/mg protein in control and 71.75±10.66 units/mg protein in treated plants.

At grain filling stage, the highest specific activity in control (72.88±0.22 units/mg protein) and in treated (86.74±0.31 units/mg protein) were observed in *Ranjit* and the lowest in control (56.00±0.28 units/mg protein) and in treated (57.91±0.30 units/mg protein) were observed in *Mahsuri*. The average specific activity was found to be 64.19±8.45 units/mg protein in control and 70.32±14.82 units/mg protein in treated plants.

At harvesting stage, the highest specific activity in control (50.23±0.25 units/mg protein) and in treated (51.02±0.34 units/mg protein) were observed in *Ranjit* and the lowest in control (45.51±0.22 units/mg protein) and in treated (45.73±0.26 units/mg protein) were observed in *Mahsuri.* The average specific activity was found to be 48.00±2.37 units/mg protein in control and 48.85±2.77 units/mg protein in treated plants.

4.7.2(a). Total activity of catalase extract prepared from brown rice

The total activity of catalase extract prepared from brown rice at two different growth stages in control and treated plants are presented in Table 4.7.2 and FIG. 4.7.2(a). The variation in the total activity of catalase of brown rice was found to be non significant regarding different levels of soil iron content.

At grain filling stage, the highest total activity in control (127.75±0.79 units/min/g fresh weight) and in treated (128.93±0.43 units/min/g fresh weight) were observed in *Ranjit* and the lowest in control (122.81±0.21 units/min/g fresh weight) and in treated (128.12±0.40 units/min/g fresh weight) were observed in *Mahsuri*. The average total activity was found to be 126.08±2.83 units/min/g fresh weight in control and 128.66±0.46 units/min/g fresh weight in treated plants.

At harvesting stage, the highest total activity in control (104.31±0.26 units/min/g fresh weight) and in treated (119.00±0.10 units/min/g fresh weight) were observed in *Ranjit* and the lowest in control (90.87±0.05 units/min/g fresh weight) and in treated (98.25±0.34 units/min/g fresh weight) were observed in *Mahsuri*. The average total activity was found to be 97.47±6.72 units/min/g fresh weight in control and 106.83±10.82 units/min/g fresh weight in treated plants.

4.7.2(b). Specific activity of catalase extract prepared from brown rice

The specific activity of catalase extract prepared from brown rice at two different growth stages in control and treated plants are presented in Table 4.7.2. and FIG. 4.7.2(b). The specific activity of catalase in brown rice at both the stages were found to be non significant irrespective of the iron content of soil.

At grain filling stage, the highest specific activity in control (7.70±0.05 units/mg protein) and in treated (8.50±0.04 units/mg protein) were observed in *Ranjit* and the lowest in control (7.48±0.09 units/mg protein) and in treated (7.64±0.07 units/mg protein) were observed in *Mahsuri*. The average specific activity was found to be 7.63±0.13 units/mg protein in control and 18.03±0.43 units/mg protein in treated plants.

At harvesting stage, the highest specific activity in control (4.33±0.03 units/mg protein) and in treated (4.76±0.04 units/mg protein) were observed in *Ranjit* and the lowest in control (4.01±0.03 units/mg protein) and in treated (4.19±0.02 units/mg protein) were observed in *Mahsuri*. The average specific activity was found to be 4.15±0.02 units/mg protein in control and 4.43±0.04 units/mg protein in treated plants.

4.8. Phytic acid P content

Phytic acid P content of brown rice at before harvesting stage in control and treated plants are presented in Table 4.8.1 and FIG. 4.8.1. It was observed that the variation between the control and treated plants were non significant.

The highest phytic acid P content in control (875.85±68.41 mg/100g) was observed in Mahsuri and in treated (997.11±43.26 mg/100g) were observed in *Kajoli chakua* and the lowest in control (647.46±55.43 mg/100g) and in treated (941.82±112.40 mg/100g) were observed in *Ranjit*. The average phytic acid P content was found to be 789.23±123.78 mg/100g in control and 975.27±29.42 mg/100g in treated plants.

4.9. pH of the soil at different stages of plant growth

pH of soil before planting and after harvesting in control and treated soils are presented in Table 4.9.1 and FIG. 4.9.1. It was observed that the variation in pH of the soil between the control and treated soils were significant both, before planting and after harvesting.

Before planting, the highest soil pH in control (5.11±0.01) was observed in *Mahsuri* and in treated (4.82±0.01) was observed in *Kajoli chakua* and the lowest in control (5.10±0.01) was observed in *Ranjit* and *Kajoli chakua* and in treated (4.80±0.01) was observed in *Ranjit* and *Mahusri.* The average soil pH was found to be 5.10±0.01 in control and 4.80±0.01 in treated soils.

After harvesting, the highest soil pH in control (6.72±0.02) was observed in *Ranjit* and in treated (6.20±0.02) was observed in *Ranjit* and *Kajoli chakua* and the lowest in control (6.52±0.02) was observed in *Kajoli chakua* and in treated (6.12±0.02) was observed in *Mahsuri*. The average soil pH was found to be 6.59±0.11 in control and 6.17±0.04 in treated soils.

4.10. Organic carbon of soil at different stages of plant growth

Organic carbon of soil before planting and after harvesting in control and treated soils are presented in Table 4.10.1. and FIG. 4.10.1. It was observed that the variation in organic carbon of soil between the control and treated soils were significant, only before planting.

Before planting, the highest organic carbon of soil in control (9.75±0.78 g/kg) was observed in *Ranjit* and in treated (11.31±0.78 g/kg) was observed in *Ranjit* and *Mahsuri* and the lowest in control (9.49±0.45 g/kg) was observed in *Mahsuri* and *Kajoli chakua* and in treated (11.18±0.59 g/kg) was observed in *Kajoli chakua.* The average organic carbon was found to be 9.57±0.15 g/kg in control and 11.26±0.07 g/kg in treated soils.

After harvesting, the highest organic carbon of soil in control (14.30±0.81 g/kg) was observed in *Mahsuri* and in treated (15.99±0.39 g/kg) was observed in *Ranjit* and the lowest in control (13.91±1.19 g/kg) was observed in *Kajoli chakua* and in treated (13.26±0.78 g/kg) was observed in *Mahsuri.* The average organic carbon was found to be 14.08±0.19 g/kg in control and 14.56±1.36 g/kg in treated soils.

4.11. Cation exchange capacity of soil at different stages of plant growth

Cation exchange capacity of soil before planting and after harvesting in control and treated soils are presented in Table 4.11.1. and FIG. 4.11.1. It was observed that the variation in cation exchange capacity of soil between the control and treated soils were significant both, before planting and after harvesting.

Before planting, the highest cation exchange capacity of soil in control [13.20±0.10 cmol $(p^+)kg^{-1}$] was observed in *Ranjit* and in treated [12.80±0.20 cmol $(p^+)kg^{-1}$] was observed in *Kajoli chakua* and the lowest in control [13.1 cmol $(p^+)kg^{-1}$] was observed in *Mahsuri* and *Kajoli chakua* and in treated [12.5 cmol $(p^+)kg^{-1}$] was observed in *Mahsuri.* The average cation exchange capacity was found to be 13.13±0.05 cmol $(p^+)kg^{-1}$ in control and 12.66±0.15 cmol $(p^+)kg^{-1}$ in treated soils.

After harvesting, the highest cation exchange capacity of soil in control [16.40±0.10 cmol $(p^+)kg^{-1}$] was observed in *Ranjit* and in treated [14.40±0.20 cmol $(p^+)kg^{-1}$] was observed in *Ranjit* and *Kajoli chakua* and the lowest in control [16.20±0.10 cmol $(p^+)kg^{-1}$] was observed in *Mahsuri* and *Kajoli chakua* and in treated [14.30±0.10 cmol $(p^+)kg^{-1}$] was observed in *Mahsuri.* The average cation exchange capacity was found to be 16.26±0.11 cmol $(p^+)kg^{-1}$ in control and 14.36±0.05 cmol $(p^+)kg^{-1}$ in treated soils.

4.12. DTPA extractable iron of soil

DTPA extractable iron of soil before planting and after harvesting in control and treated soils are presented in Table 4.12.1. and FIG. 4.12.1. It was observed that the variation in DTPA extractable iron of soil between the control and treated soils were significant both, before planting and after harvesting.

Before planting, the highest DTPA extractable iron of soil in control (160.00±2.00 mg/kg) and in treated (182.03±0.97 mg/kg) were observed in *Mahsuri* and the lowest in control (159.10±0.90 mg/kg) was observed in *Ranjit* and *Kajoli chakua* and in treated (182.01± 0.99 mg/kg) was observed in *Ranjit.* The average DTPA extractable iron was found to be 159.40±0.51 mg/kg in control and 182.35±0.57 mg/kg in treated soils.

After harvesting, the highest DTPA extractable iron of soil in control (118.25±0.75 mg/kg) and in treated (144.75±0.25 mg/kg) were observed in *Ranjit* and the lowest in control (112.08mg/kg) was observed in *Ranjit* and *Kajoli chakua* and in treated (139.53 mg/kg) was observed in *Mahsuri.* The average DTPA extractable iron was found to be 115.84±3.30 mg/kg in control and 142.25±2.61 mg/kg in treated soils.

Table 4.1.1 Moisture content (%, fresh weight basis) of rice leaves at three different growth stages

Variety/ Stage	Maximum tillering stage		Grain filling stage		Harvesting stage	
	Control	**Treated**	**Control**	**Treated**	**Control**	**Treated**
Ranjit	69.11±1.53	70.17±0.25	72.94±0.07	72.84±0.02	8.78±0.20	10.01±0.10
Mahsuri	65.06±0.13	67.55±0.38	71.46±0.01	65.87±0.02	9.83±0.02	7.15±0.01
Kajoli chakua	75.50±0.20	82.36±0.15	72.97±0.02	73.16±0.03	7.26±0.07	8.83±0.17
Mean	69.89±5.26	73.36±7.90	72.45±0.86	70.62±4.11	8.62±1.29	8.66±1.43
t value		-1.98		0.97		-0.02
P value		0.18 NS		0.43 NS		0.97 NS
SE (m)	3.03	4.56	0.49	2.37	0.74	0.82

N B : The data represented are the mean of three replications ± Standard deviation

NS : Not Significant

Table 4.1.2. Moisture content (%, fresh weight basis) of paddy and brown rice at two different growth stages

Variety/ Stage	Grain filling stage		Harvesting stage	
	Control	**Treated**	**Control**	**Treated**
Ranjit	13.76±0.40 (11.24±0.04)	13.60±0.20 (11.36±0.02)	13.33±0.30 (10.11±0.06)	12.73±0.11 (10.43±0.09)
Mahsuri	12.93±0.11 (11.60±0.02)	11.33±0.30 (11.28±0.04)	13.93±0.05 (10.91±0.07)	14.06±0.23 (10.73±0.02)
Kajoli chakua	13.53±0.50 (12.29±0.05)	13.26±0.50 (11.98±0.06)	12.73±0.23 (11.61±0.04)	12.06±0.11 (11.44±0.04)
Mean	13.40±0.42 (11.71±0.53)	12.73±1.22 (11.54±0.38)	13.33±0.60 (10.87±0.75)	12.95±1.01 (10.86±0.52)
t value		1.46 (1.17)		1.48 (0.06)
P value		0.28NS (0.36 NS)		0.27 NS (0.95 NS)
SE (m)	0.24 (0.31)	0.70 (0.22)	0.34 (0.43)	0.58 (0.30)

NB: The data represented are the mean of three replications ± Standard deviation

The data in the parentheses represent the corresponding values for brown rice

NS : Not significant

Table 4.2.1. Total chlorophyll content (mg/g, fresh weight) of rice leaves at three different growth stages

Variety/ Stage	Maximum tillering stage		Grain filling stage		Harvesting stage	
	Control	**Treated**	**Control**	**Treated**	**Control**	**Treated**
Ranjit	1.24±0.02	0.94±0.01	2.24±0.02	1.78±0.02	0.44±0.02	0.31±0.02
Mahsuri	1.17±0.02	0.88±0.02	2.07±0.02	1.73±0.03	0.38±0.01	0.29±0.02
Kajoli chakua	1.28±0.02	1.01±0.02	2.33±0.03	1.93±0.02	0.57±0.01	0.49±0.04
Mean	1.23±0.05	0.94±0.06	2.21±0.13	1.81±0.10	0.46±0.09	0.36±0.10
t value		46.86		12.14		6.36
P value		0.00*		0.01*		0.02*
SE (m)	0.03	0.04	0.08	0.06	0.05	0.06

N B : The data represented are the mean of three replications ± Standard deviation

*Significant at 5% level of probability

Table 4.3.1. Ash content (%, dry weight basis) of rice leaves at three different growth stages

Variety/ Stage	Maximum tillering stage		Grain filling stage		Harvesting stage	
	Control	Treated	Control	Treated	Control	Treated
Ranjit	12.00±0.91	14.84±0.96	14.25±1.65	14.72±0.08	20.93±0.12	16.08±0.08
Mahsuri	14.48±0.08	15.04±0.08	14.45±0.04	12.32±0.08	18.56±0.08	19.73±0.46
Kajoli chakua	14.50±0.55	15.46±0.61	16.10±0.12	12.29±0.12	17.81±0.20	17.33±0.12
Mean	13.66±1.43	15.11±0.31	14.93±1.01	13.15±1.35	19.10±1.62	17.71±1.85
t value		-2.06		1.46		0.77
P value		0.17 NS		0.27 NS		0.52 NS
SE (m)	0.83	0.18	0.58	0.78	0.94	1.07

N B : The data represented are the mean of three replications ± Standard deviation

NS : Not significant

Table 4.3.2. Ash content (%, dry weight basis) of brown rice at grain filling stage

Variety/ Stage	Grain filling stage	
	Control	**Treated**
Ranjit	1.70±0.02	1.54±0.00
Mahsuri	2.33±0.11	1.87±0.12
Kajoli chakua	1.89±0.10	1.53±0.11
Mean	1.97±0.32	1.65±0.19
t value		3.69
P value		0.06 NS
SE (m)	0.18	0.11

N B : The data represented are the mean of three replications ± Standard deviation

NS : Not significant

Table 4.3.3. Ash content (%, dry weight basis) of brown rice at harvesting stage

Variety / Grain	TPR		TSR		MPR		MSR		BPR		BSR	
position	C	T	C	T	C	T	C	T	C	T	C	T
Ranjit	1.94	1.82	2.00	1.77	1.97	1.82	1.86	1.78	2.40	1.81	1.82	1.96
	±0.04	±0.02	±0.40	±0.06	±0.02	±0.02	±0.08	±0.06	±0.40	±0.02	±0.04	±0.04
Mahsuri	1.80	1.72	1.97	1.84	1.61	1.52	2.09	1.66	1.92	1.68	1.60	1.61
	±0.31	±0.04	±0.02	±0.04	±0.02	±0.28	±0.10	±0.04	±0.08	±0.04	±0.06	±0.02
Kajoli	1.76	1.63	1.70	1.63	1.90	1.76	1.83	1.60	1.90	1.86	1.80	1.73
chakua	±0.05	±0.15	±0.10	±0.23	±0.10	±0.15	±0.05	±0.34	±0.10	±0.05	±0.10	±0.73
Mean	1.83	1.72	1.89	1.74	1.82	1.70	1.93	1.68	2.07	1.78	1.75	1.76
	±0.09	±0.09	±0.16	±0.10	±0.19	±0.16	±0.14	±0.09	±0.28	±0.09	±0.09	±0.17
t value		6.96		3.06		7.66		2.45		1.77		-0.14
P value		0.02*		0.09 NS		0.58 NS		0.13 NS		0.21 NS		0.89 NS
SE(m)	0.05	0.05	0.09	0.02	0.10	0.09	0.08	0.05	0.16	0.05	0.05	0.10

N B : The data represented are the mean of three replications ± Standard deviation

TPR : Top primary rachis **MPR :** Middle primary rachis **BPR :** Bottom primary rachis

TSR : Top secondary rachis **MSR :** Middle secondary rachis **BSR :** Bottom secondary rachis

C : Control, **T :** Treated, *Significant at 5% level of probability, NS : Not significant

Table 4.3.4. Ash content (%, dry weight basis) of rice husk at two different growth stages

Variety/ Stage	Grain filling stage		Harvesting stage	
	Control	**Treated**	**Control**	**Treated**
Ranjit	14.52±0.04	13.42±0.04	14.11±0.15	12.07±0.04
Mahsuri	12.53±0.22	10.28±0.06	12.47±0.16	11.39±0.22
Kajoli chakua	13.50±0.14	11.11±0.06	13.20±0.43	12.52±0.12
Mean	13.51±0.99	11.60±1.62	13.26±0.82	11.99±0.56
t value		4.68		3.13
P value		0.04*		0.08 NS
SE (m)	0.57	0.93	0.47	0.32

N B : The data represented are the mean of three replications ± Standard deviation

*Significant at 5% level of probability

NS: Not significant

The results represent mean for the cumulative samples irrespective of the position on the panicle

Table 4.3.5. Comparison of ash content (%, dry weight basis) of brown rice and rice husk at harvesting stage

Variety/ Stage	Brown rice		Rice husk	
	Control	**Treated**	**Control**	**Treated**
Ranjit	1.99±0.20	1.82±0.06	14.11±0.15	12.07±0.04
Mahsuri	1.83±0.19	1.67±0.10	12.47±0.16	11.39±0.22
Kajoli chakua	1.81±0.07	1.73±0.09	13.20±0.43	12.52±0.12
Mean	1.87±0.09	1.74±0.07	13.26±0.82	11.99±0.56
t value		4.79		3.13
P value		0.04*		0.08 NS
SE (m)	0.05	0.04	0.47	0.32

The data represented are the mean of three replications ± Standard deviation

*Significant at 5% level of probability

NS : Not significant

The results represent mean for the cumulative samples irrespective of the position on the panicle

Table 4.4.1. Iron content (mg/100g, dry weight basis) of rice leaves at three different growth stages

Variety/ Stage	Maximum tillering stage		Grain filling stage		Harvesting stage	
	Control	**Treated**	**Control**	**Treated**	**Control**	**Treated**
Ranjit	27.64±0.06	54.17±0.25	58.81±0.26	75.01±0.26	57.58±0.01	69.71±0.19
Mahsuri	16.12±0.20	33.91±0.03	18.39±0.12	36.76±0.12	24.77±0.21	26.75±0.14
Kajoli chakua	27.29±0.36	36.01±0.04	47.05±0.03	62.26±0.07	35.12±0.06	39.62±0.20
Mean	23.68±6.55	41.36±11.14	41.42±20.79	58.01±19.47	39.16±16.77	45.36±22.04
t value		-3.43		-17.78		-2.03
P value		0.07 NS		0.00*		0.17 NS
SE (m)	3.78	6.43	12.00	11.24	9.68	12.72

The data represented are the mean of three replications ± Standard deviation

*Significant at 5% level of probability

NS : Not significant

Table 4.4.2. Iron content (mg/100g, dry weight basis) of brown rice at grain filling stage

Variety/ Stage	Grain filling stage	
	Control	**Treated**
Ranjit	2.18±0.08	5.92±0.10
Mahsuri	1.17±0.04	4.18±0.03
Kajoli chakua	1.87±0.14	5.13±0.08
Mean	1.74±5.17	5.08±8.70
t value		-15.57
P value		0.00*
SE (m)	0.29	0.50

The data represented are the mean of three replications ± Standard deviation

*Significant at 5% level of probability

Table 4.4.3. Iron content (mg/100g, dry weight basis) of brown rice at harvesting stage

Variety / Grain	TPR		TSR		MPR		MSR		BPR		BSR	
position	C	T	C	T	C	T	C	T	C	T	C	T
Ranjt	5.18	11.09	4.85	10.62	4.77	10.54	4.04	10.26	3.91	10.21	3.76	9.67
	±0.15	±0.22	±0.08	±0.13	±0.15	±0.26	±0.08	±0.36	±0.11	±0.32	±0.11	±0.17
Mahsuri	3.26	7.94	3.04	6.89	2.71	6.77	2.28	6.73	1.97	6.01	1.86	5.88
	±0.09	±0.01	±0.05	±0.07	±0.04	±0.11	±0.11	±0.06	±0.13	±0.07	±0.05	±0.06
Kajoli chakua	3.86	8.37	3.60	8.34	3.53	8.02	3.36	7.85	3.25	7.66	3.21	7.48
	±0.16	±0.17	±0.19	±0.12	±0.14	±0.24	±0.07	±0.14	±0.09	±0.15	±0.07	±0.01
Mean	4.10	9.13	3.83	8.61	3.67	8.44	3.23	8.28	3.04	7.96	2.94	7.68
	±0.98	±1.70	±0.92	±1.87	±1.03	±1.92	±0.89	±1.80	±0.98	±2.11	±0.98	±1.89
t value		-11.46		-8.64		-9.29		-8.66		-7.02		-7.98
P value		0.00*		0.01*		0.01*		0.01*		0.01*		0.01*
SE (m)	0.56	0.98	0.53	1.08	0.59	1.10	0.51	1.04	0.56	1.22	0.56	1.09

The data represented are the mean of three replications ± Standard deviation

TPR : Top primary rachis **MPR :** Middle primary rachis **BPR :** Bottom primary rachis

TSR : Top secondary rachis **MSR :** Middle secondary rachis **BSR :** Bottom secondary rachis

C : Control, **T :** Treated, *Significant at 5% level of probability

Table 4.4.4. Iron content (mg/100g, dry weight basis) of rice husk at two different growth stages

Variety/ Stage	Grain filling stage		Harvesting stage	
	Control	**Treated**	**Control**	**Treated**
Ranjit	5.50±0.30	9.37±0.01	8.91±0.01	12.07±0.30
Mahsuri	3.11±0.02	6.96±0.10	6.95±0.18	11.17±0.32
Kajoli chakua	3.68±0.01	8.91±0.10	7.96±0.58	11.46±0.22
Mean	4.09±1.24	8.41±1.27	7.94±0.98	11.56±0.45
t value		-9.45		-11.60
P value		0.01*		0.01*
SE (m)	0.72	0.73	0.56	0.26

N B: The data represented are the mean of three replications ± Standard deviation

*Significant at 5% level of probability

The results represent mean for the cumulative samples irrespective of the position on the panicle

Table 4.4.5. Comparison of iron content (mg/100g, dry weight basis) of brown rice and rice husk at harvesting stage

Variety/ Stage	Brown rice		Rice husk	
	Control	**Treated**	**Control**	**Treated**
Ranjit	4.49±0.55	10.39±0.47	8.91±0.01	12.07±0.30
Mahsuri	2.25±0.57	6.70±0.73	6.95±0.18	11.17±0.32
Kajoli chakua	3.46±0.24	7.95±0.36	7.96±0.58	11.46±0.22
Mean	3.40±1.12	8.34±1.87	7.94±0.98	11.56±0.45
t value		-10.37		-11.60
P value		0.00*		0.01*
SE (m)	0.64	1.08	0.56	0.26

The data represented are the mean of three replications ± Standard deviation

*Significant at 5% level of probability

NS : Not significant

The results represent mean for the cumulative samples irrespective of the position on the panicle

Table 4.5.1. Total activity (units/min/g fresh weight) and specific activity (units/mg protein) of peroxidase extract prepared from rice leaves at three different growth stages

Variety/ Stage		Maximum tillering stage		Grain filling stage		Harvesting stage	
		Control	Treated	Control	Treated	Control	Treated
Ranjit	**TA**	21.14±0.04	21.86±0.11	21.74±0.03	21.96±0.02	22.64±0.04	23.30±0.03
	SA	50.34±1.10	78.09±2.63	53.04±1.23	59.36±1.24	38.37±1.25	55.49±1.19
Mahsuri	**TA**	22.81±0.01	23.12±0.07	22.46±0.03	23.00±0.20	24.02±0.03	22.82±0.03
	SA	49.59±1.05	57.80±1.36	49.92±2.20	60.52±1.31	42.15±1.13	43.88±1.10
Kajoli	**TA**	22.30±0.08	22.80±0.04	22.80±0.05	22.22±0.01	23.62±0.05	24.14±0.04
chakua	**SA**	48.47±1.36	65.14±1.15	54.28±1.41	55.55±1.37	36.33±1.24	44.70±1.16
Mean	**TA**	22.08±0.85	22.59±0.65	22.33±0.54	22.39±0.54	23.42±0.71	23.42±0.66
	SA	49.46±0.94	67.01±10.27	52.41±2.24	58.47±2.60	38.95±2.95	48.02±6.47
t value	**TA**		-4.30		-0.18		0.01
	SA		-3.10		-2.24		-2.03
P value	**TA**		0.05*		0.87 NS		0.99 NS
	SA		0.09 NS		0.15 NS		0.17 NS
SE (m)	**TA**	0.49	0.37	0.31	0.31	0.40	0.38
	SA	0.54	5.93	1.29	1.50	1.70	3.74

N B : The data represented are the mean of three replications ± Standard deviation; *Significant at 5% level of probability; NS : Not significant; TA : Total activity, SA: Specific activity

Table 4.5.2. Total activity (units/min/g fresh weight) and specific activity (units/mg protein) of peroxidase extract prepared from brown rice at two different growth stages

Variety/ Stage		Grain filling stage		Harvesting stage	
		Control	Treated	Control	Treated
Ranjit	**TA**	19.33±0.31	20.26±0.24	20.76±0.12	21.76±0.32
	SA	2.91±0.02	3.61±0.05	2.10±0.01	3.84±0.04
Mahsuri	**TA**	20.04±0.51	20.86±0.22	21.79±0.33	22.10±0.14
	SA	2.50±0.01	2.89±0.03	2.21±0.02	2.03±0.02
Kajoli chakua	**TA**	20.06±0.44	20.47±0.52	21.02±0.42	21.65±0.61
	SA	2.99±0.01	3.30±0.01	1.90±0.01	2.32±0.05
Mean	**TA**	19.81±0.41	20.53±0.30	21.19±0.53	21.50±0.35
	SA	2.80±0.26	3.26±0.36	2.07±0.15	2.73±0.97
t value	**TA**		-4.55		-0.61
	SA		-3.92		-1.16
P value	**TA**		0.04*		0.60 NS
	SA		0.05*		0.36 NS
SE (m)	**TA**	0.24	0.17	0.30	0.20
	SA	0.15	0.20	0.09	0.56

N.B: The data represented are the mean of three replications; *Significant at 5% level of probability; NS : Not significant; TA : Total activity; SA: Specific activity

Table 4.6.1. Total activity (units/min/g fresh weight) and specific activity (units/mg protein) of superoxide dismutase extract prepared from rice leaves at three different growth stages

Variety/ Stage		Maximum tillering stage		Grain filling stage		Harvesting stage	
		Control	Treated	Control	Treated	Control	Treated
Ranjit	**TA**	0.75±0.05	1.25±0.01	1.25±0.03	1.50±0.01	1.25±0.02	1.50±0.01
	SA	2.34±0.22	2.60±0.31	3.04±0.20	4.05±0.32	2.45±0.21	4.05±0.32
Mahsuri	**TA**	0.50±0.03	1.00±0.10	0.50±0.02	1.00±0.05	0.25±0.01	0.50±0.03
	SA	1.61±0.19	2.50±0.24	1.85±0.21	2.63±0.30	0.55±0.22	1.04±0.21
Kajoli	**TA**	0.50±0.01	0.75±0.03	1.00±0.05	1.50±0.03	0.75±0.03	1.25±0.03
chakua	**SA**	1.13±0.20	1.59±0.22	3.12±0.20	3.65±0.22	1.87±0.30	3.28±0.20
Mean	**TA**	0.58±0.14	1.00±0.25	0.91±0.38	1.33±0.28	0.75±0.50	1.08±0.52
	SA	1.69±0.60	2.23±0.55	2.67±0.71	3.44±0.73	1.62±0.97	2.79±1.56
t value	**TA**		-5.00		-5.00		-4.00
	SA		-2.88		-5.57		-3.40
P value	**TA**		0.03*		0.03*		0.05*
	SA		0.10 NS		0.03*		0.07 NS
SE (m)	**TA**	0.08	0.14	0.22	0.16	0.28	0.30
	SA	0.35	0.32	0.41	0.42	0.56	0.90

N.B: The data represented are the mean of three replications; *Significant at 5% level of probability; NS : Not significant; TA : Total activity; SA: Specific activity

Table 4.6.2. Total activity (units/min/g fresh weight) and specific activity (units/mg protein) of superoxide dismutase extract prepared from brown rice at two different growth stages

Variety/ Stage		Grain filling stage		Harvesting stage	
		Control	Treated	Control	Treated
Ranjit	**TA**	1.50±0.02	1.00±0.05	0.75±0.01	1.50±0.03
	SA	1.72±0.01	1.92±0.02	0.98±0.02	2.20±0.03
Mahsuri	**TA**	0.50±0.01	1.25±0.03	0.25±0.03	1.50±0.01
	SA	0.57±0.03	1.27±0.03	0.25±0.01	1.63±0.02
Kajoli chakua	**TA**	0.75±0.02	1.50±0.01	1.00±0.02	1.25±0.02
	SA	0.88±0.01	1.97±0.02	0.83±0.04	1.28±0.02
Mean	**TA**	0.91±0.52	1.25±0.25	0.66±0.38	1.41±0.14
	SA	1.05±0.59	1.72±0.39	0.68±0.38	1.70±0.02
t value	**TA**		-0.80		-2.59
	SA		-2.57		-3.54
P value	**TA**		0.50 NS		0.12 NS
	SA		0.12 NS		0.07 NS
SE (m)	**TA**	0.30	0.14	0.22	0.08
	SA	0.34	0.22	0.22	0.26

N.B: The data represented are the mean of three replications; NS: Not significant; TA : Total activity; SA: Specific activity

Table 4.7.1. Total activity (units/min/g fresh weight) and specific activity (units/mg protein) of catalase extract prepared from rice leaves at three different growth stages

Variety/ Stage		Maximum tillering stage		Grain filling stage		Harvesting stage	
		Control	Treated	Control	Treated	Control	Treated
Ranjit	**TA**	88.81±0.24	106.62±0.21	107.87±0.52	114.50±0.48	80.37±0.44	84.18±0.22
	SA	71.62±0.32	80.16±0.40	72.88±0.22	86.74±0.31	50.23±0.25	51.02±0.34
Mahsuri	**TA**	79.50±0.69	92.62±0.32	84.56±0.23	80.50±0.09	74.18±0.14	76.37±0.18
	SA	53.71±0.29	59.75±0.26	56.00±0.28	57.91±0.30	45.51±0.22	45.73±0.26
Kajoli	**TA**	109.37±0.60	122.06±1.38	83.43±0.22	97.50±0.35	78.68±0.11	85.18±0.53
chakua	**SA**	68.78±0.20	75.34±0.36	63.69±0.31	66.32±0.36	48.27±0.30	49.81±0.24
Mean	**TA**	92.56±15.28	107.10±14.72	91.95±13.79	97.50±17.00	77.74±3.19	81.91±4.82
	SA	64.70±9.62	71.75±10.66	64.19±8.45	70.32±14.82	48.00±2.37	48.85±2.77
t value	**TA**		-8.86		-1.05		-3.31
	SA		-9.25		-1.58		-2.22
P value	**TA**		0.01*		0.40 NS		0.08 NS
	SA		0.01*		0.25 NS		0.15 NS
SE (m)	**TA**	8.82	8.50	7.96	9.81	1.84	2.78
	SA	5.55	6.15	4.87	8.55	1.36	1.60

N.B: The data represented are the mean of three replications; *Significant at 5% level of probability; NS : Not significant; TA : Total activity; SA: Specific activity

Table 4.7.2. Total activity (units/min/g fresh weight) and specific activity (units/mg protein) of catalase extract prepared from brown rice at two different growth stages

Variety/ Stage		Grain filling stage		Harvesting stage	
		Control	Treated	Control	Treated
Ranjit	**TA**	127.75±0.79	128.93±0.43	104.31±0.26	119.00±0.10
	SA	7.70±0.05	8.50±0.04	4.33±0.03	4.76±0.04
Mahsuri	**TA**	122.81±0.21	128.12±0.40	90.87±0.05	98.25±0.34
	SA	7.48±0.09	7.64±0.07	4.01±0.03	4.19±0.02
Kajoli chakua	**TA**	127.68±0.50	128.93±0.23	97.25±0.23	103.25±0.46
	SA	7.73±0.04	7.97±0.04	4.12±0.02	4.34±0.03
Mean	**TA**	126.08±2.83	128.66±0.46	97.47±6.72	106.83±10.82
	SA	7.63±0.13	8.03±0.43	4.15±0.02	4.43±0.04
t value	**TA**		-1.89		-3.47
	SA		-1.98		-3.56
P value	**TA**		0.19 NS		0.07 NS
	SA		0.18 NS		0.07 NS
SE (m)	**TA**	1.63	0.27	3.88	6.25
	SA	0.07	0.25	0.09	0.17

N.B: The data represented are the mean of three replications; NS: Not significant; TA : Total activity; SA: Specific activity

Table 4.8.1. Phytic acid P content (mg/100g, dry weight basis) of brown rice at harvesting stage

Variety/ Stage	Harvesting stage	
	Control	**Treated**
Ranjit	647.46±55.43	941.82±112.40
Mahsuri	875.85±68.41	986.90±32.20
Kajoli chakua	844.38±73.41	997.11±43.26
Mean	789.23±123.78	975.27±29.42
t value		-3.35
P value		0.07 NS
SE (m)	71.46	16.98

N B : The data represented are the mean of three replications ± Standard deviation

NS : Not significant

Table 4.9.1. pH of the soil at different stages of plant growth

Variety	Before planting		After harvesting	
	Control	Treated	Control	Treated
Ranjit	5.10±0.01	4.80±0.01	6.72±0.02	6.20±0.02
Mahsuri	5.11±0.01	4.80±0.01	6.53±0.02	6.12±0.02
Kajoli chakua	5.10±0.01	4.82±0.01	6.52±0.02	6.20±0.02
Mean	5.10±0.01	4.80±0.01	6.59±0.11	6.17±0.04
t value		33.63		7.20
P value		0.00*		0.01*
SE (m)	0.00	0.01	0.06	0.02

N B : The data represented are the mean of three replications ± Standard deviation

*Significant at 5% level of probability

Table 4.10.1. Organic carbon content (g/kg) of soil at different stages of plant growth

Variety	Before planting		After harvesting	
	Control	**Treated**	**Control**	**Treated**
Ranjit	9.75±0.78	11.31±0.78	14.04±0.78	15.99±0.39
Mahsuri	9.49±0.45	11.31±0.78	14.30±0.81	13.26±0.78
Kajoli chakua	9.49±0.59	11.18±0.59	13.91±1.19	14.43±0.78
Mean	9.57±0.15	11.26±0.07	14.08±0.19	14.56±1.36
t value		-22.51		-0.55
P value		0.00*		0.63 NS
SE (m)	0.08	0.04	0.11	0.79

N B : The data represented are the mean of three replications ± Standard deviation

*Significant at 5% level of probability

NS : Not significant

Table 4.11.1. Cation Exchange Capacity (CEC) [cmol (p^+) kg^{-1}] of soil at different stages of plant growth

Variety	Before planting		After harvesting	
	Control	Treated	Control	Treated
Ranjit	13.20±0.10	12.70±0.20	16.40±0.10	14.40±0.20
Mahsuri	13.10±0.20	12.50±0.20	16.20±0.10	14.30±0.10
Kajoli chakua	13.10±0.20	12.80±0.20	16.20±0.10	14.40±0.20
Mean	13.13±0.05	12.66±0.15	16.26±0.11	14.36±0.05
t value		5.29		32.90
P value		0.03*		0.00*
SE (m)	0.03	0.08	1.90	1.51

N B : The data represented are the mean of three replications ± Standard deviation

*Significant at 5% level of probability

Table 4.12. 1. DTPA extractable iron (mg/kg) of soil at different stages of plant growth

Variety	Before planting		After harvesting	
	Control	Treated	Control	Treated
Ranjit	159.10±0.90	182.01± 0.99	118.25±0.75	144.75±0.25
Mahsuri	160.00±2.00	182.03±0.97	112.08±0.92	139.53±0.47
Kajoli chakua	159.10±0.90	183.01±0.04	117.20±0.80	142.48±1.52
Mean	159.40±0.51	182.35±0.57	115.84±3.30	142.25±2.61
Difference		22.95		26.41
t value		-42.25		-41.84
P value		0.00*		0.00*
SE (m)	0.30	0.33	1.90	1.51

N B : The data represented are the mean of three replications ± Standard deviation

*Significant at 5% level of probability

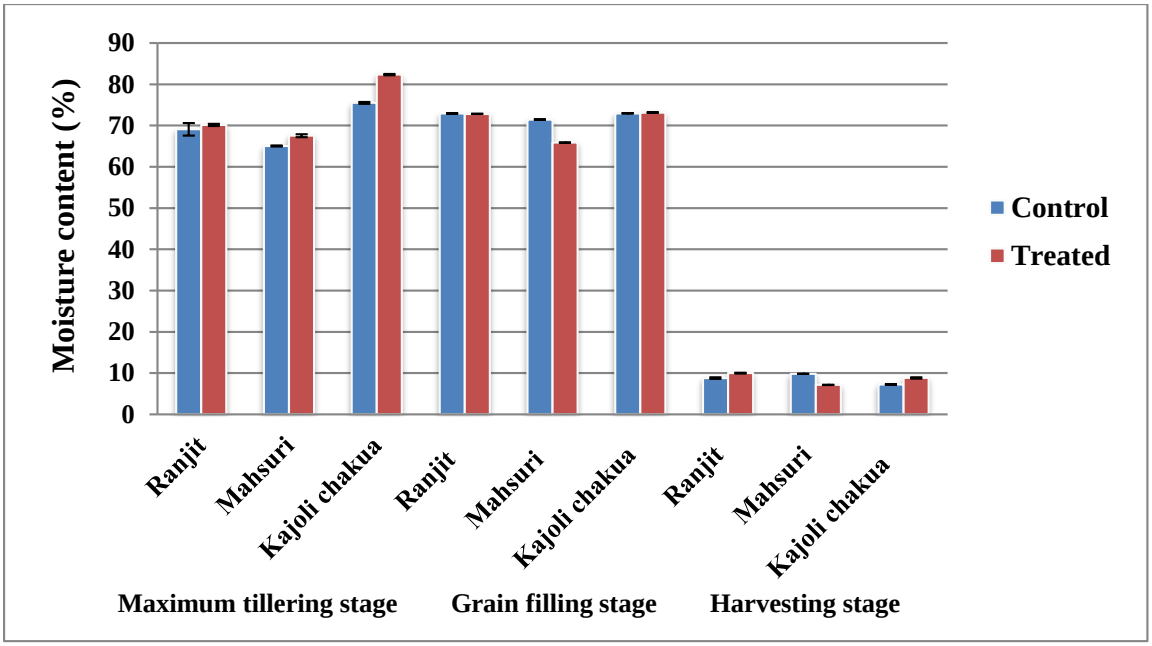


FIG.4.1.1. MOISTURE CONTENT (%, FRESH WEIGHT BASIS) OF RICE LEAVES AT THREE DIFFERENT GROWTH STAGES

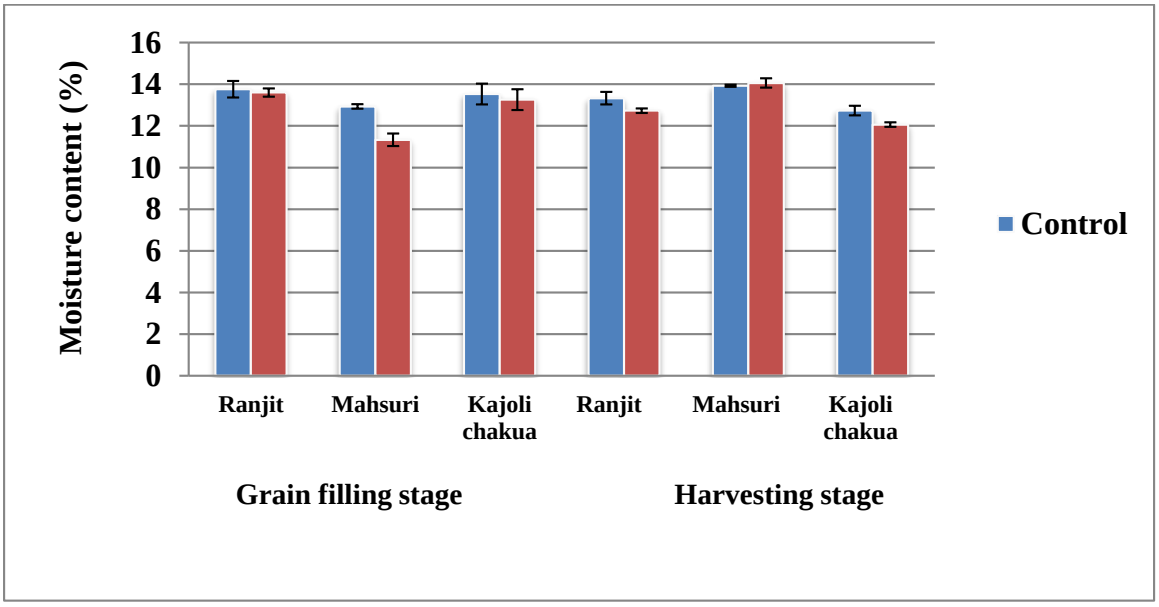


FIG.4.1.2 (a). MOISTURE CONTENT (%, FRESH WEIGHT BASIS) OF PADDY AT TWO DIFFERENT GROWTH STAGES

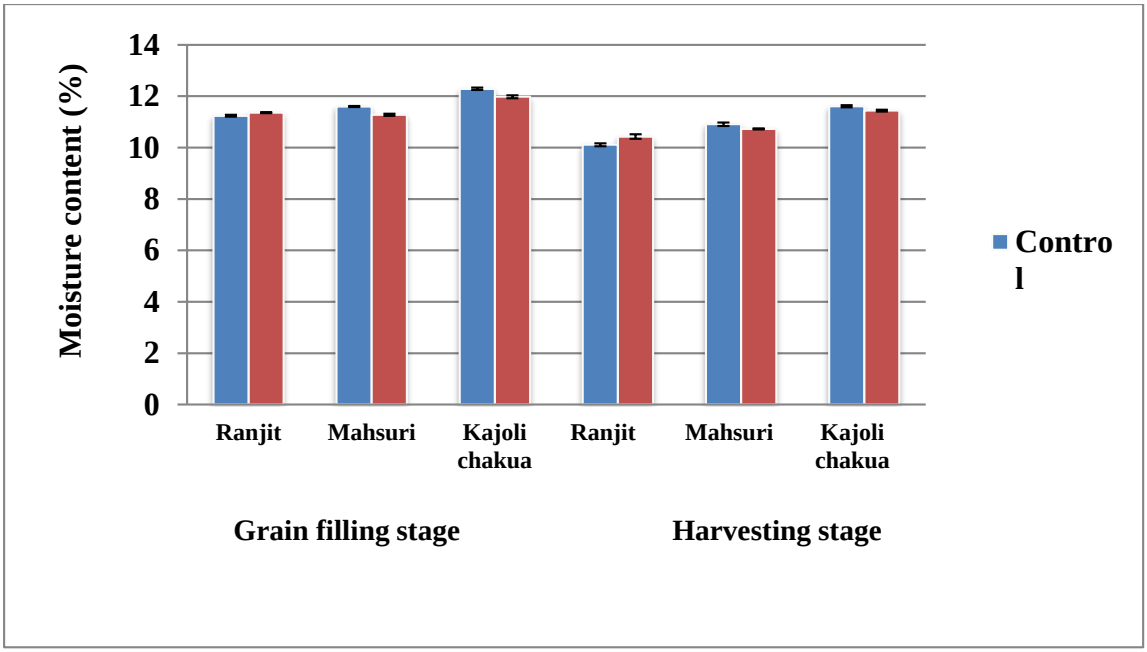


FIG.4.1.2 (b). MOISTURE CONTENT (%, FRESH WEIGHT BASIS) OF BROWN RICE AT TWO DIFFERENT GROWTH STAGES

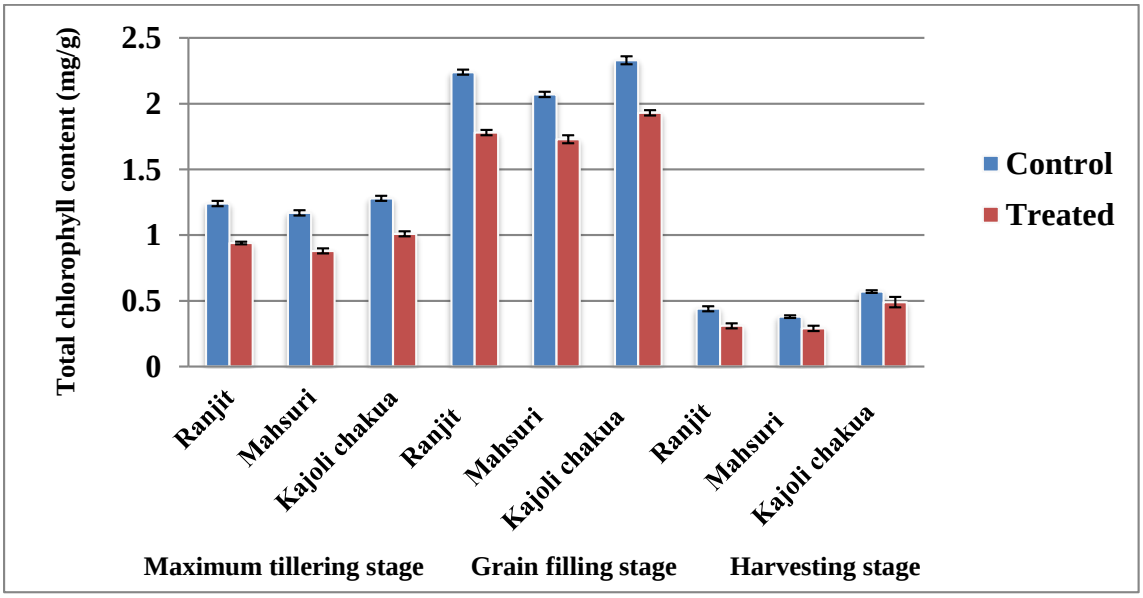


FIG.4.2.1. TOTAL CHLOROPHYLL CONTENT (mg/g, FRESH WEIGHT BASIS) OF RICE LEAVES AT THREE DIFFERENT GROWTH STAGES

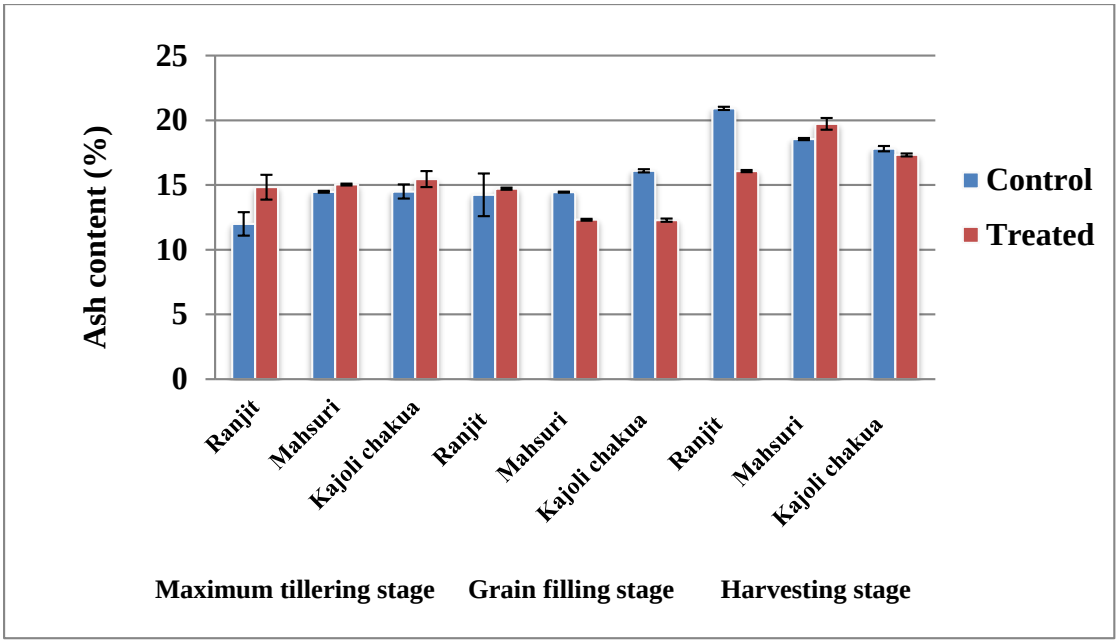


FIG.4.3.1. ASH CONTENT (%, DRY WEIGHT BASIS) OF RICE LEAVES AT THREE DIFFERENT GROWTH STAGES

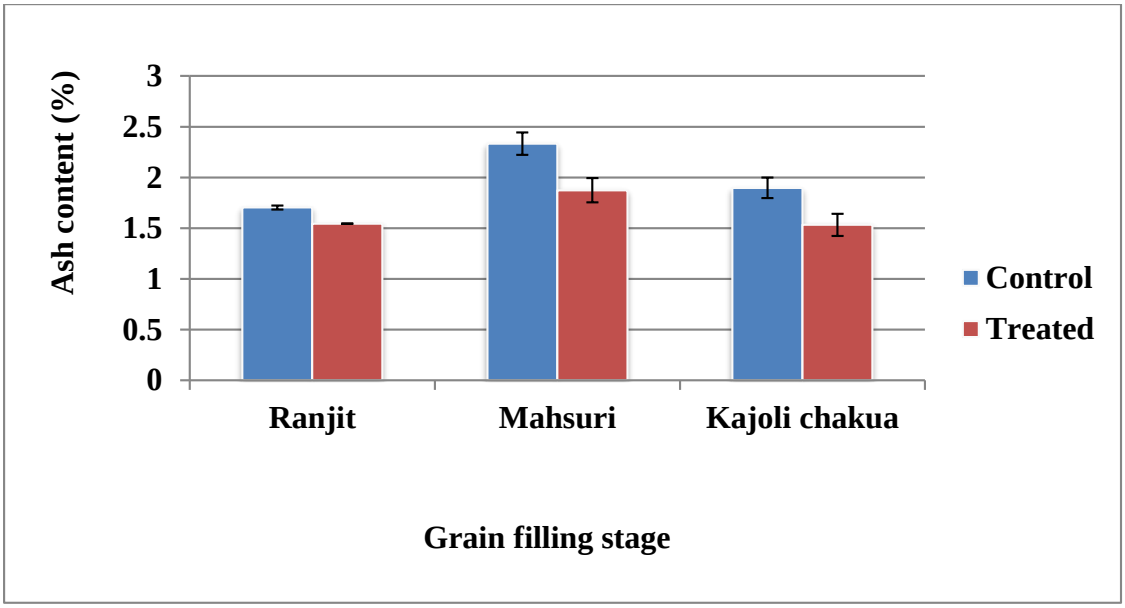


FIG.4.3.2. ASH CONTENT (%, DRY WEIGHT BASIS) OF BROWN RICE AT GRAIN FILLING STAGE

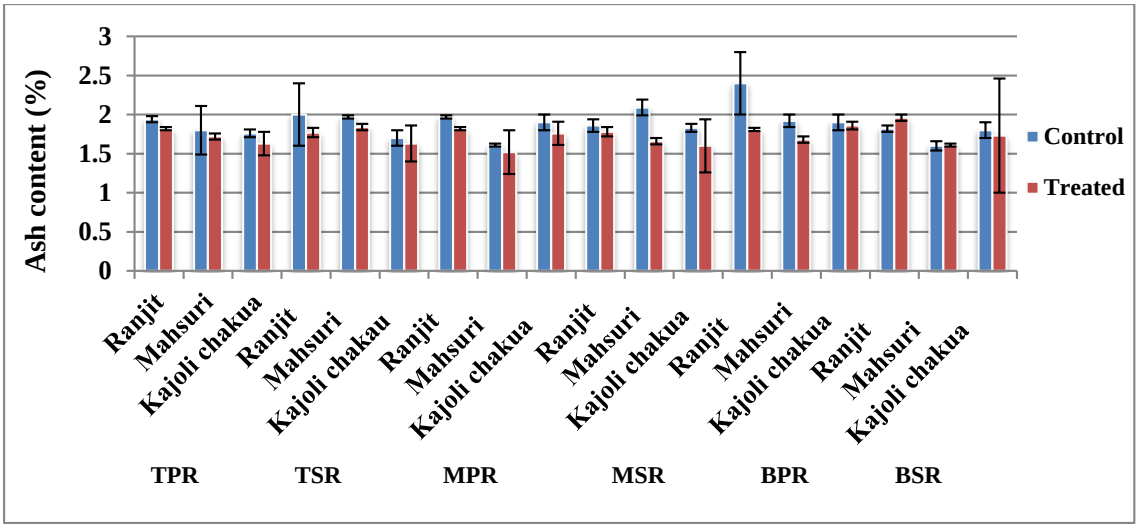


FIG.4.3.3. ASH CONTENT (%, DRY WEIGHT BASIS) OF BROWN RICE AT HARVESTING STAGE

N B: TPR: Top primary rachis, TSR: Top secondary rachis, MPR: Middle primary rachis, MSR: Middle secondary rachis, BPR: Bottom primary rachis, BSR: Bottom secondary rachis

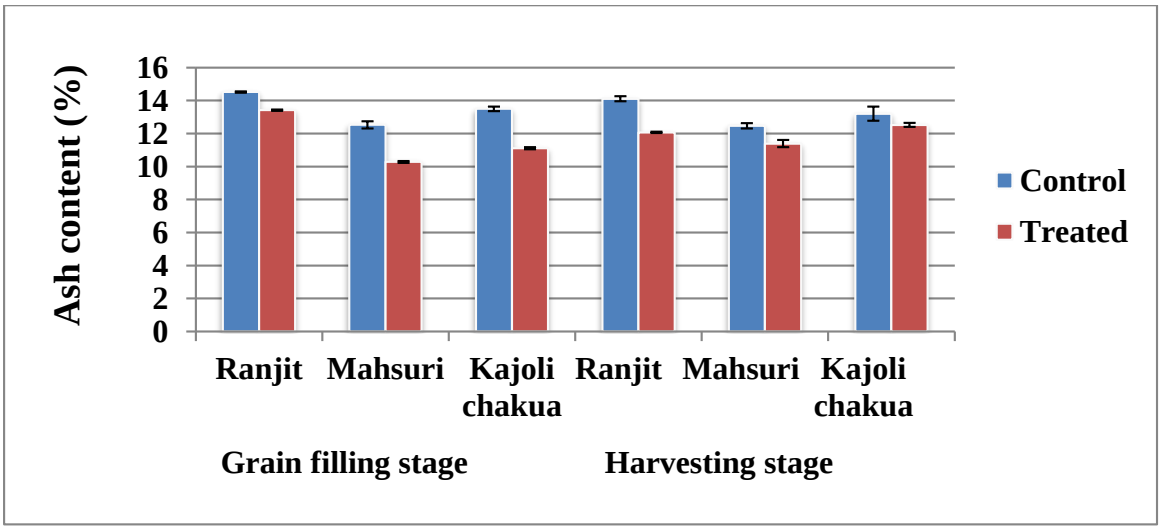


FIG.4.3.4. ASH CONTENT (%, DRY WEIGHT BASIS) OF RICE HUSK AT TWO DIFFERENT GROWTH STAGES

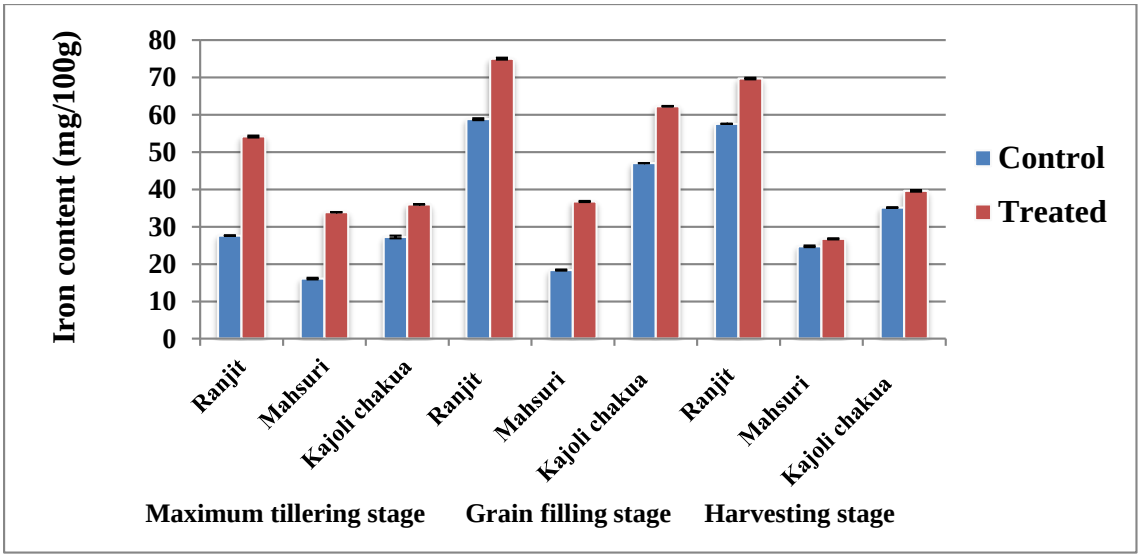


FIG.4.4.1. IRON CONTENT (mg/100g, DRY WEIGHT BASIS) OF RICE LEAVES AT THREE DIFFERENT GROWTH STAGES

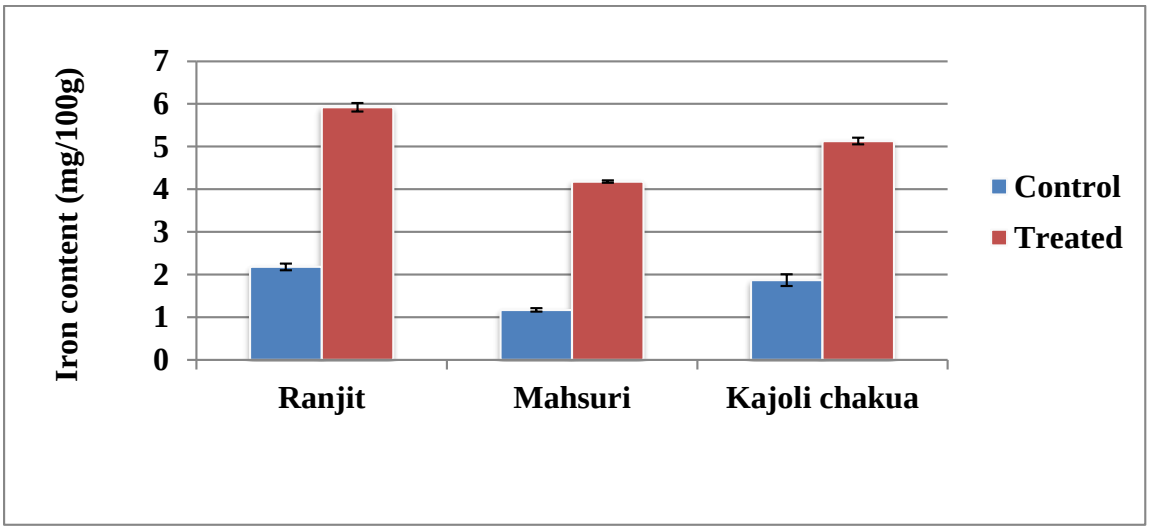


FIG.4.4.2. IRON CONTENT (mg/100g, DRY WEIGHT BASIS) OF BROWN RICE AT GRAIN FILLING STAGE

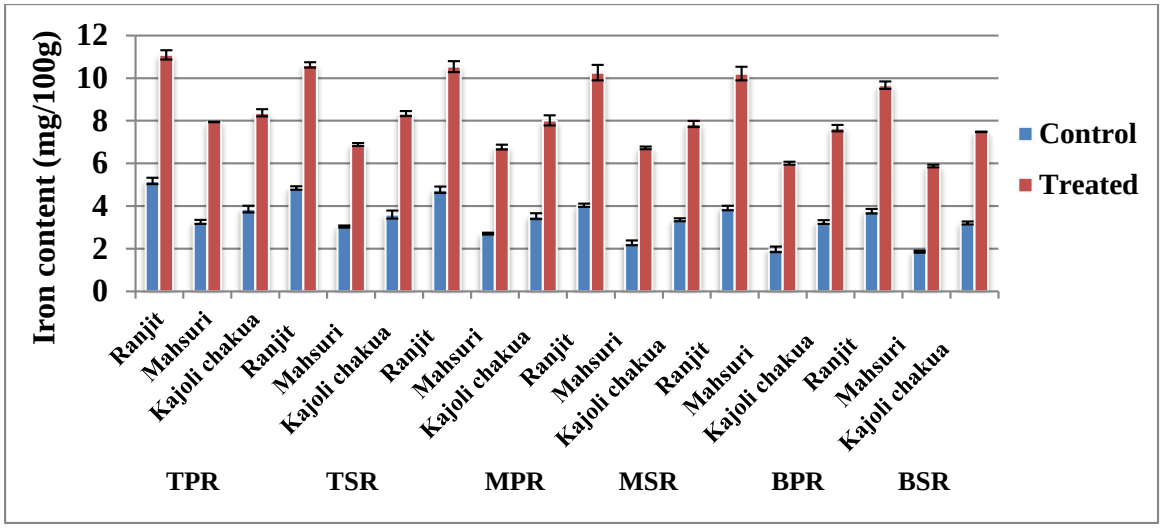


FIG.4.4.3. IRON CONTENT (mg/100g, DRY WEIGHT BASIS) OF BROWN RICE AT HARVESTING STAGE

N B: TPR: Top primary rachis, TSR: Top secondary rachis, MPR: Middle primary rachis, MSR: Middle secondary rachis, BPR: Bottom primary rachis, BSR: Bottom secondary rachis

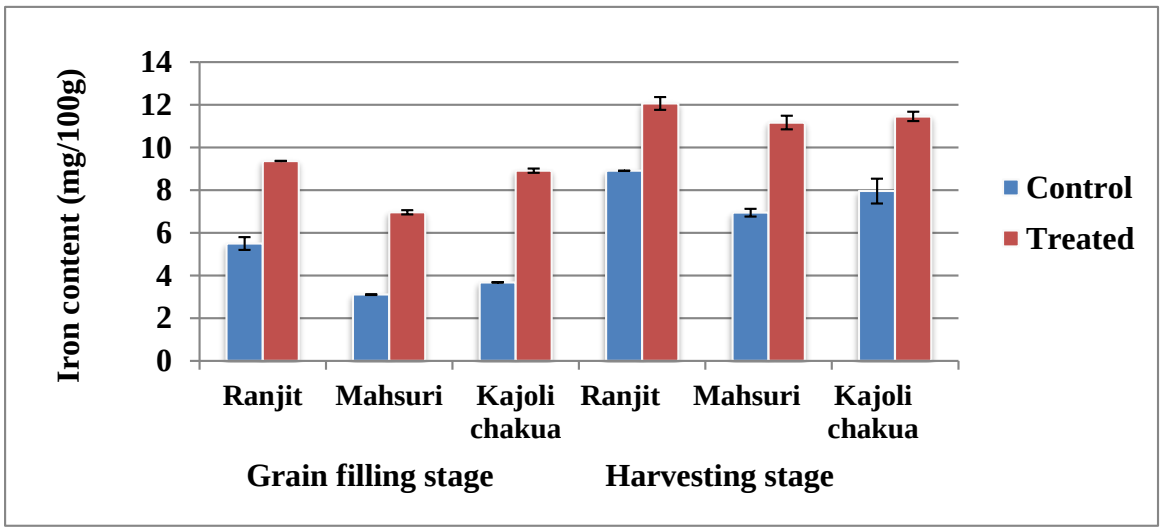


FIG.4.4.4. IRON CONTENT (mg/100g, DRY WEIGHT BASIS) OF RICE HUSK AT TWO DIFFERENT GROWTH STAGES

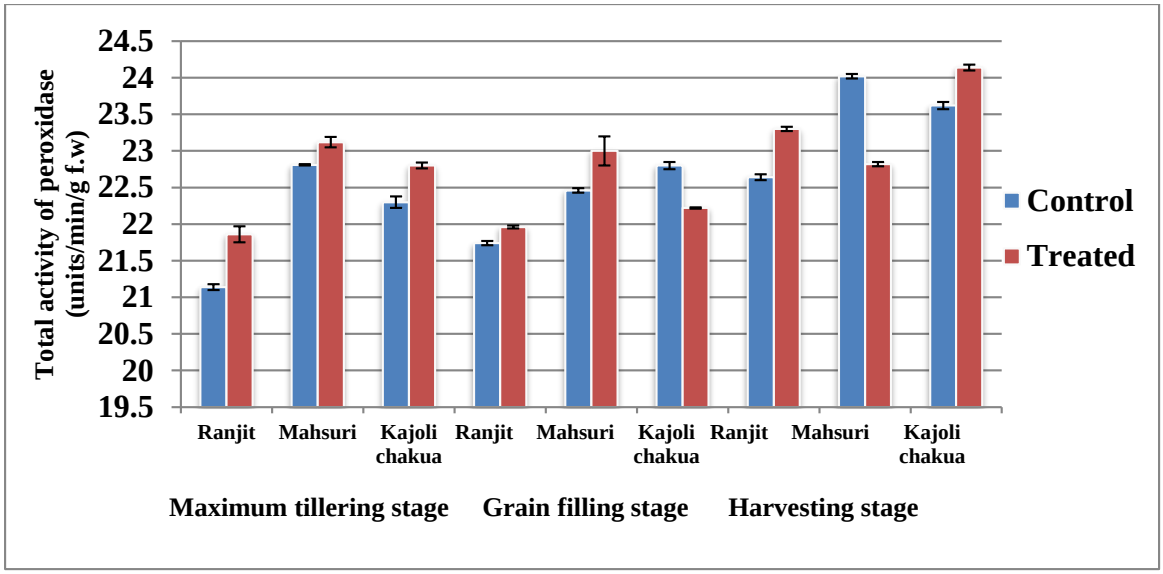


FIG.4.5.1(a). TOTAL ACTIVITY (units/min/g fresh weight) OF PEROXIDASE EXTRACT PREPARED FROM RICE LEAVES AT THREE DIFFERENT GROWTH STAGES

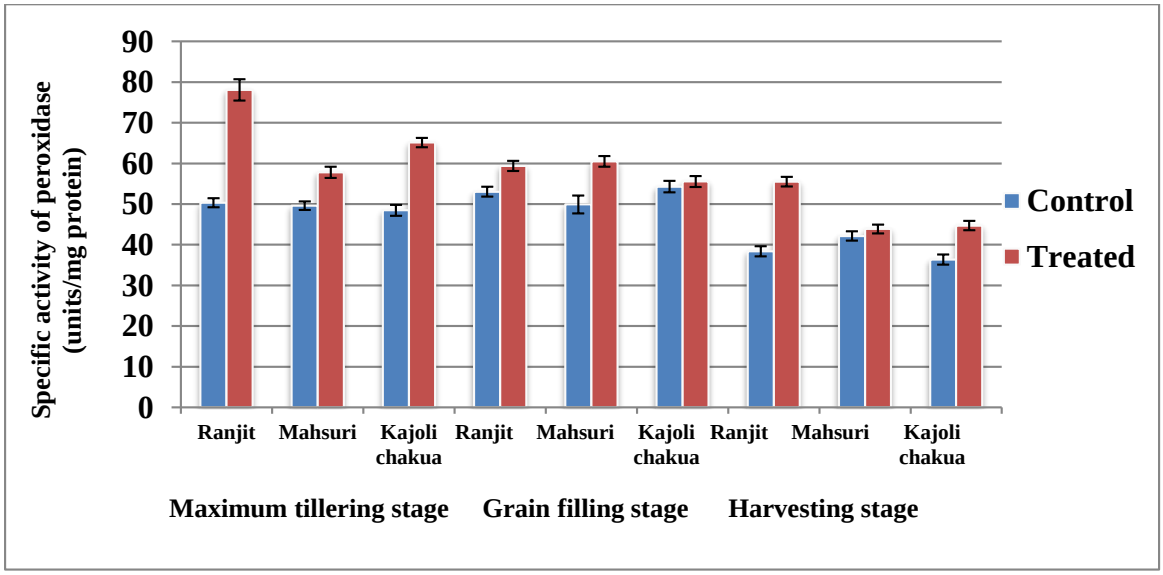


FIG.4.5.1(b). SPECIFIC ACTIVITY (units/mg protein) OF PEROXIDASE EXTRACT PREPARED FROM RICE LEAVES AT THREE DIFFERENT GROWTH STAGES

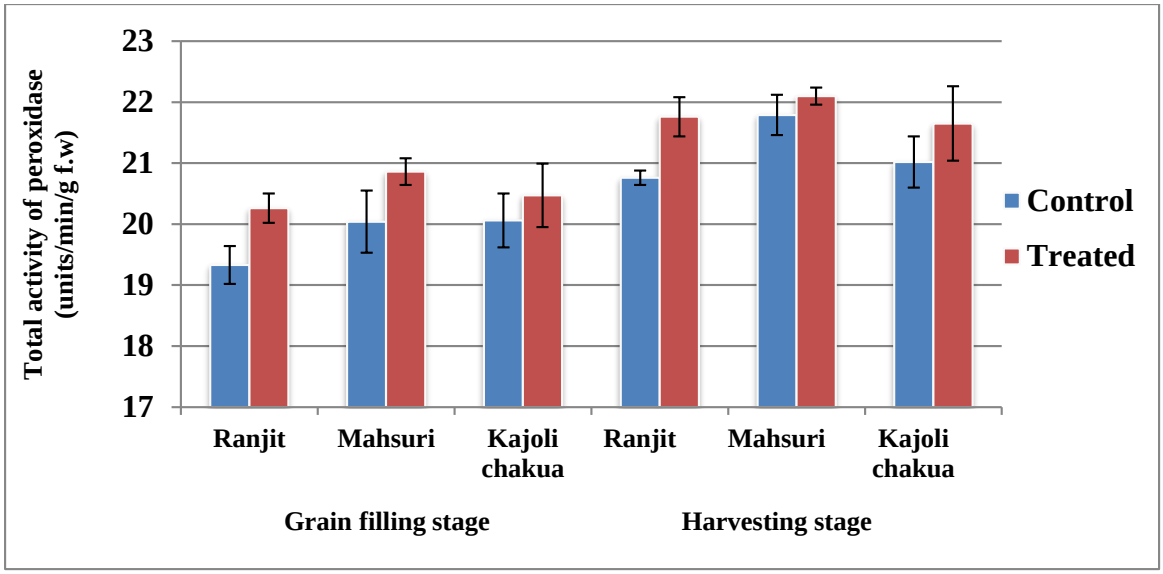


FIG.4.5.2(a). TOTAL ACTIVITY (units/min/g fresh weight) OF PEROXIDASE EXTRACT PREPARED FROM BROWN RICE AT TWO DIFFERENT GROWTH STAGES

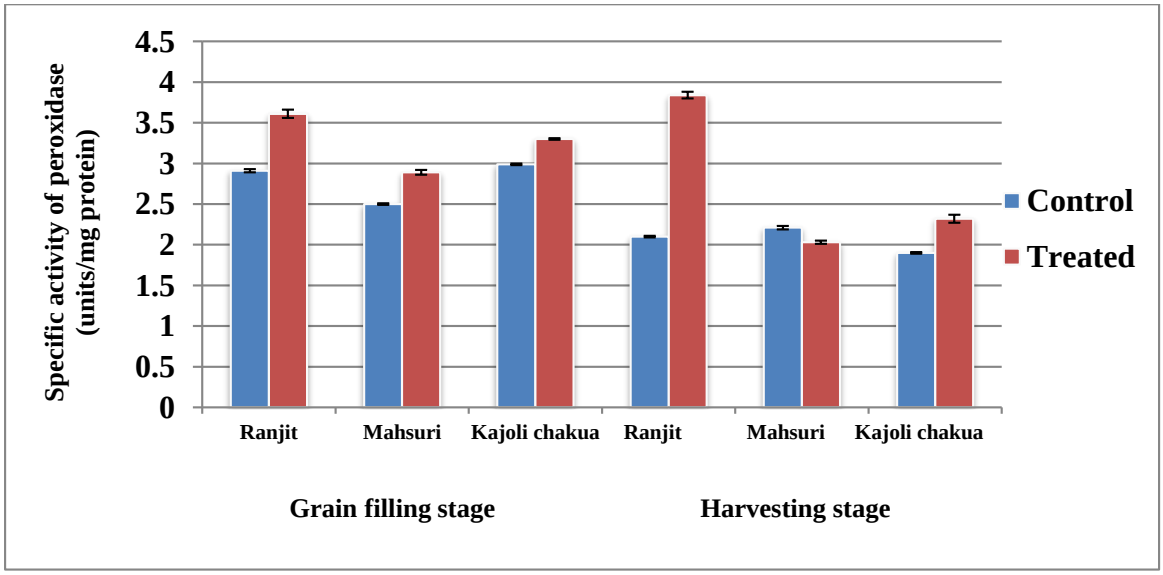


FIG.4.5.2(b). SPECIFIC ACTIVITY (units/mg protein) OF PEROXIDASE EXTRACT PREPARED FROM BROWN RICE AT TWO DIFFERENT GROWTH STAGES

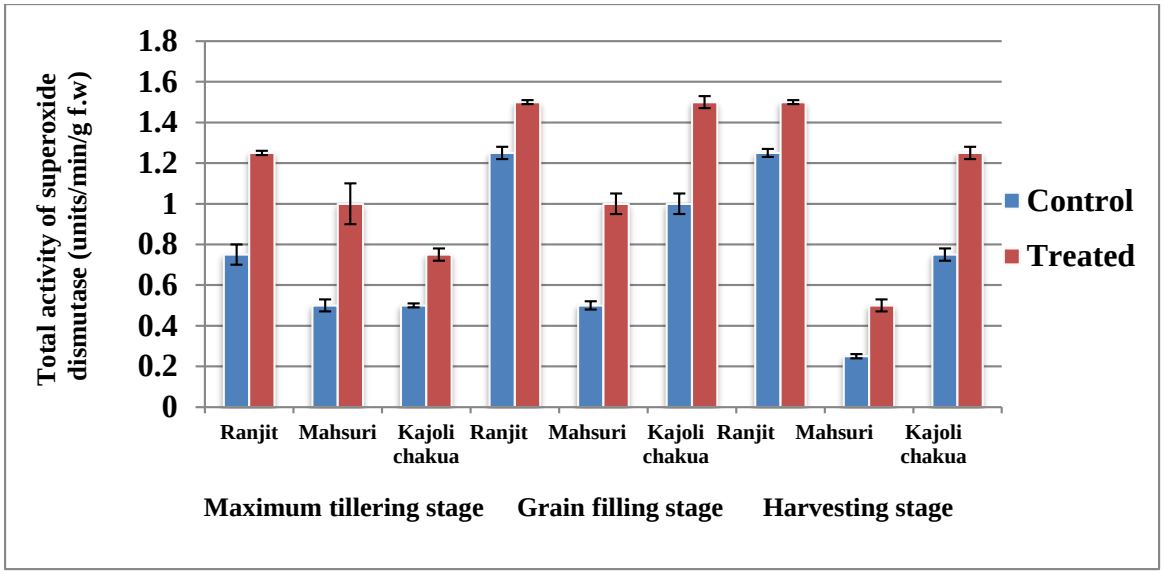


FIG.4.6.1(a). TOTAL ACTIVITY (units/min/g fresh weight) OF SUPEROXIDE DISMUTASE EXTRACT PREPARED FROM RICE LEAVES AT THREE DIFFERENT GROWTH STAGES

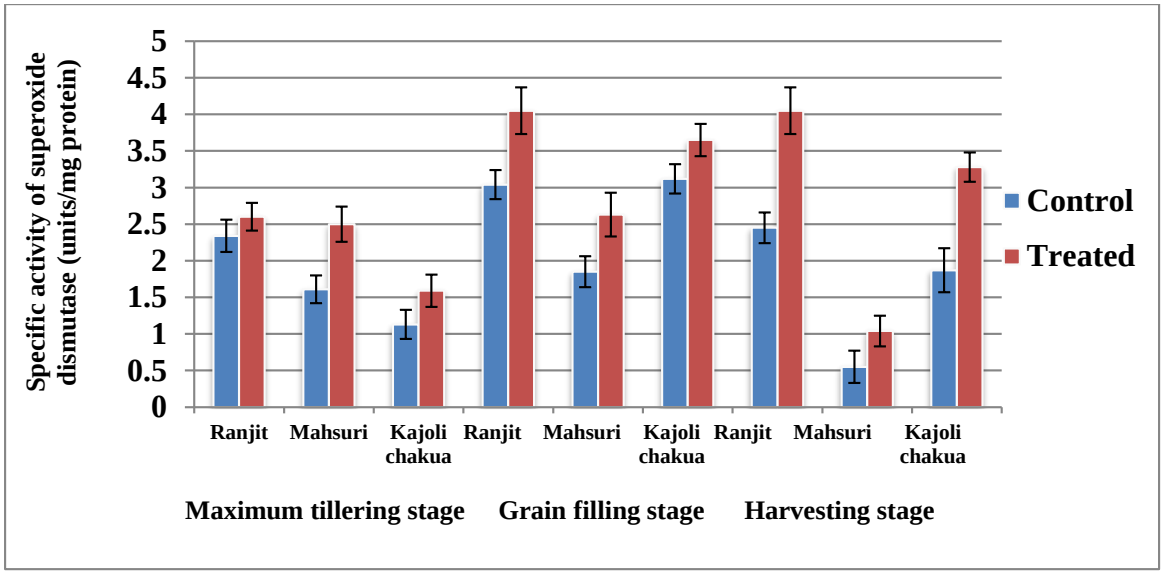


FIG.4.6.1(b). SPECIFIC ACTIVITY (units/mg protein) OF SUPEROXIDE DISMUTASE EXTRACT PREPARED FROM RICE LEAVES AT THREE DIFFERENT GROWTH STAGES

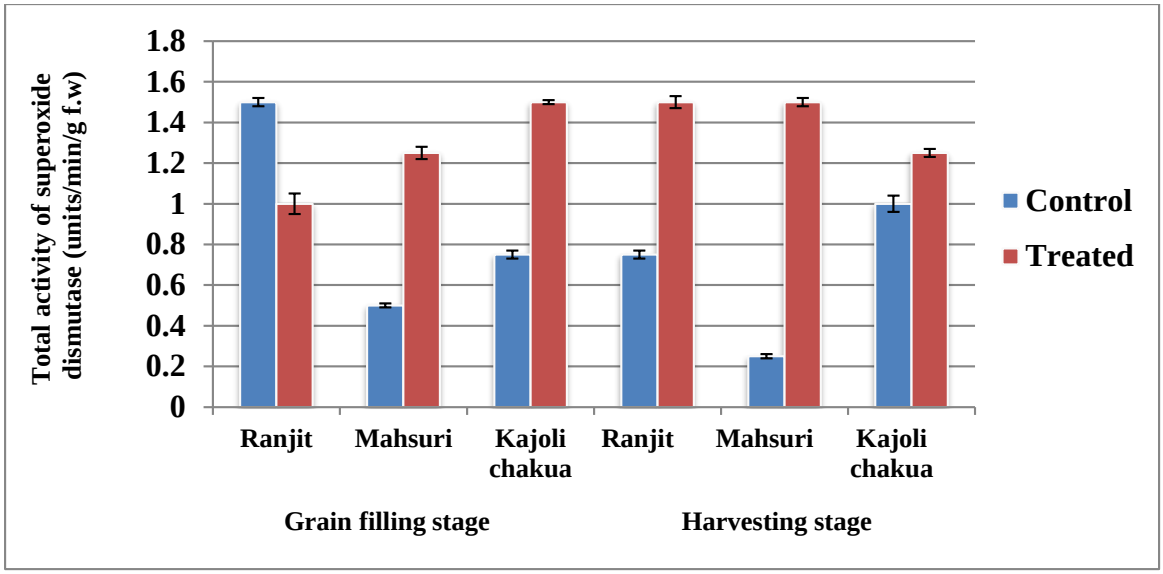


FIG.4.6.2(a). TOTAL ACTIVITY (units/min/g fresh weight) OF SUPEROXIDE DISMUTASE EXTRACT PREPARED FROM BROWN RICE AT TWO DIFFERENT GROWTH STAGES

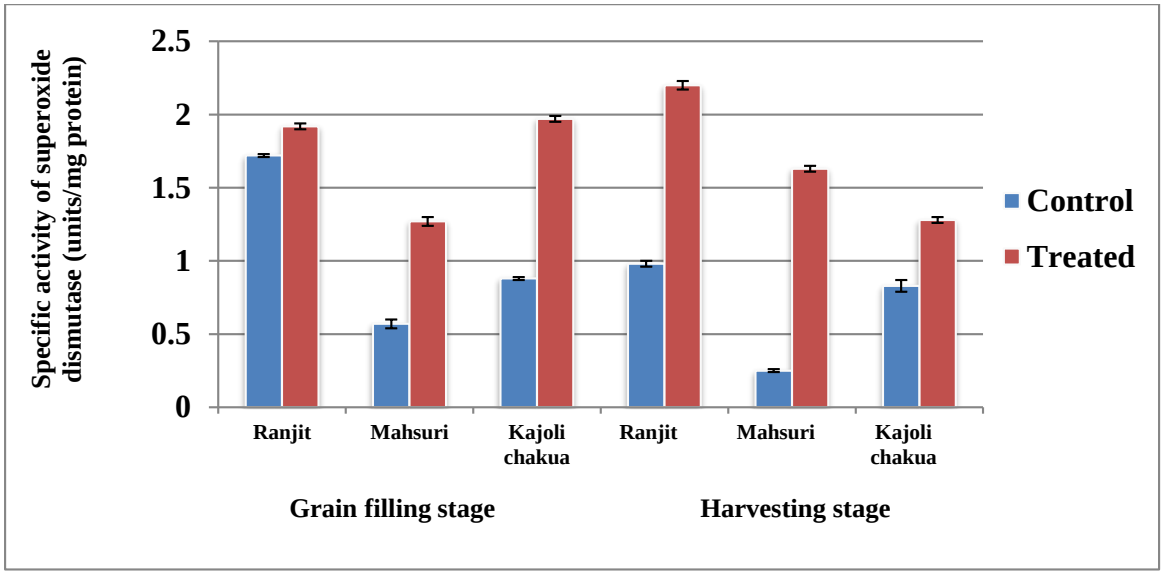


FIG.4.6.2(b). SPECIFIC ACTIVITY (units/mg protein) OF SUPEROXIDE DISMUTASE EXTRACT PREPARED FROM BROWN RICE AT TWO DIFFERENT GROWTH STAGES

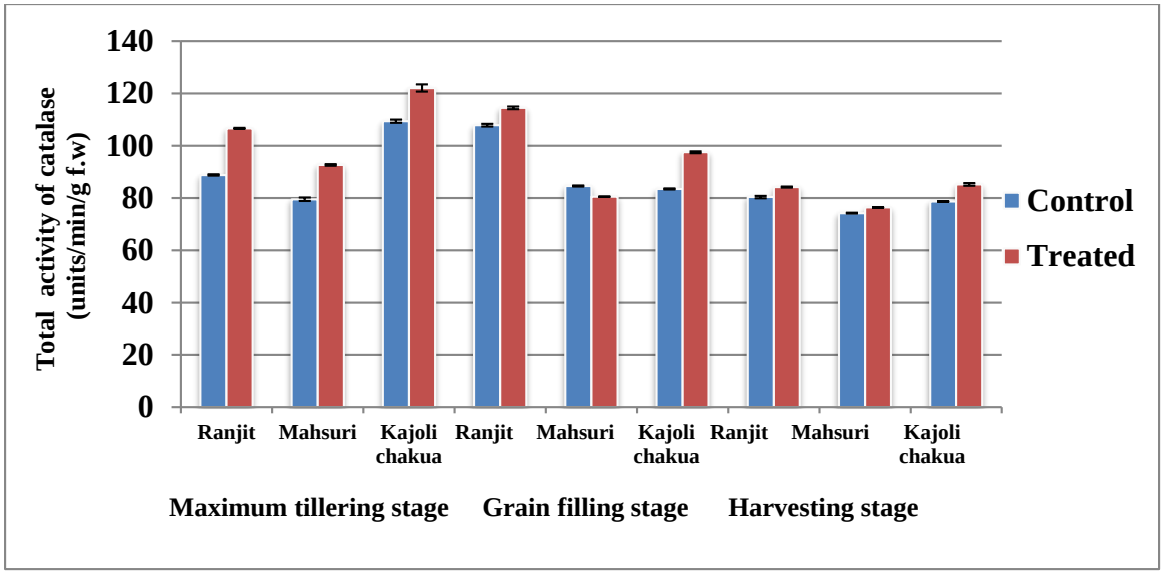


FIG.4.7.1(a). TOTAL ACTIVITY (units/min/g fresh weight) OF CATALASE EXTRACT PREPARED FROM RICE LEAVES AT THREE DIFFERENT GROWTH STAGES

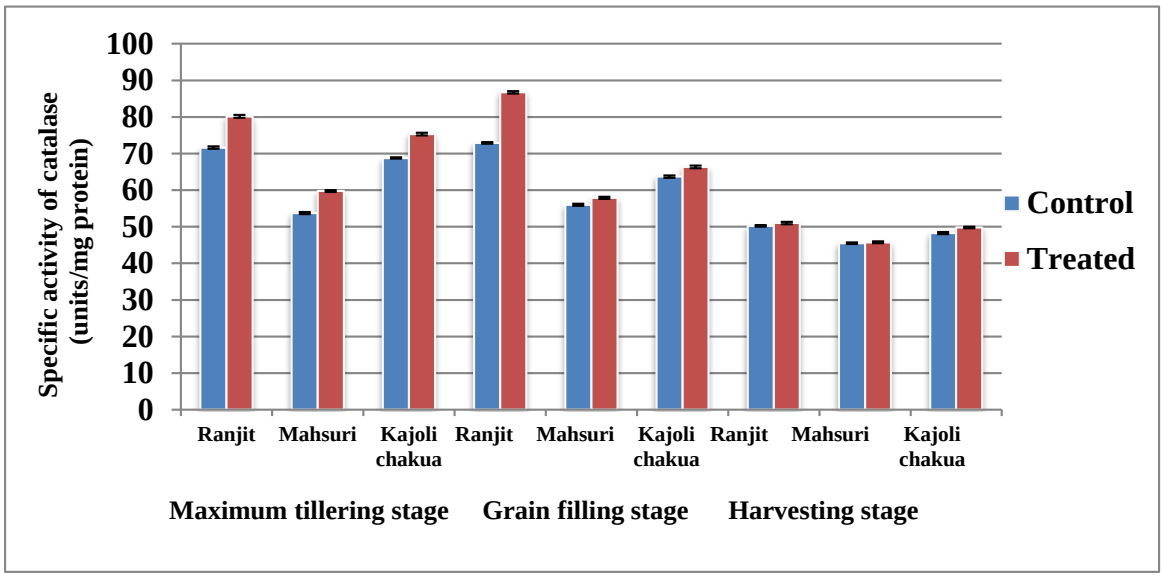


FIG.4.7.1(b). SPECIFIC ACTIVITY (units/mg protein) OF CATALASE EXTRACT PREPARED FROM RICE LEAVES AT THREE DIFFERENT GROWTH STAGES

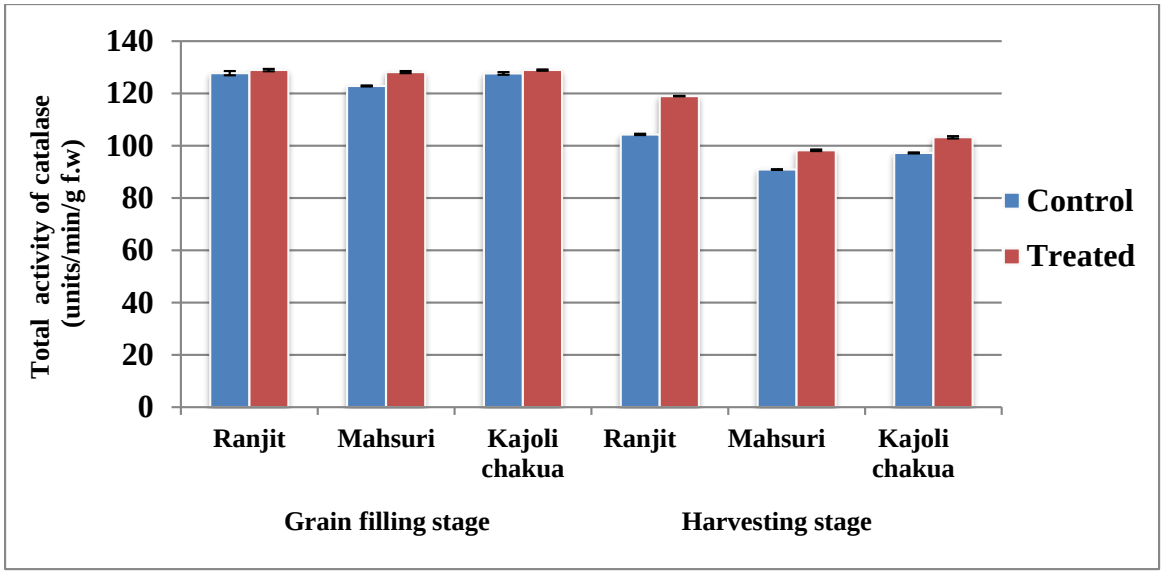


FIG.4.7.2(a). TOTAL ACTIVITY (units/min/g fresh weight) OF CATALASE EXTRACT PREPARED FROM BROWN RICE AT TWO DIFFERENT GROWTH STAGES

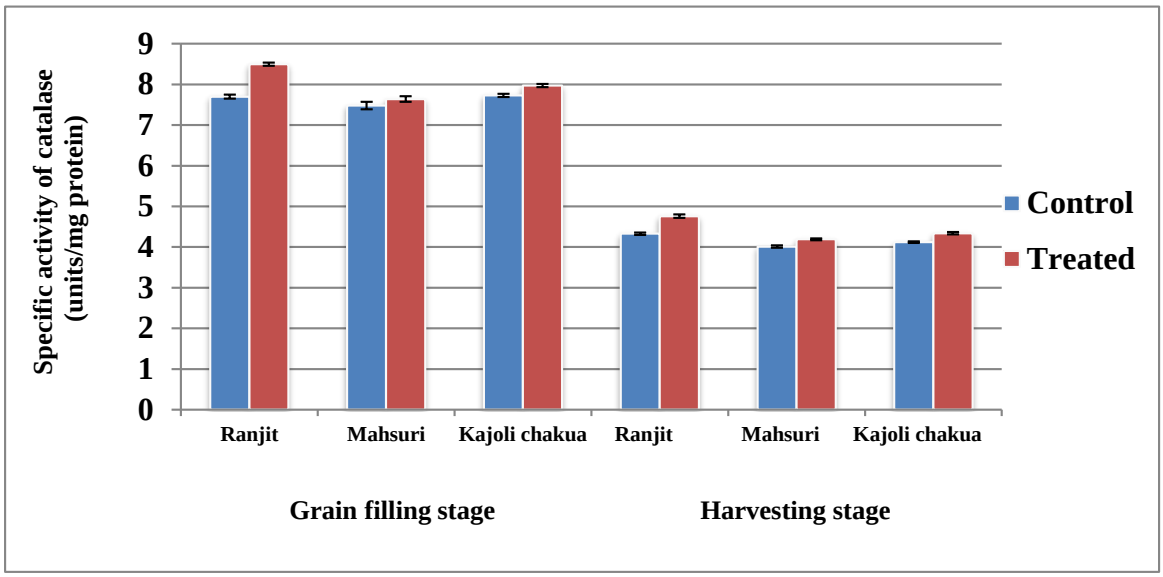


FIG.4.7.2(b). SPECIFIC ACTIVITY (units/mg protein) OF CATALASE EXTRACT PREPARED FROM BROWN RICE AT TWO DIFFERENT GROWTH STAGES

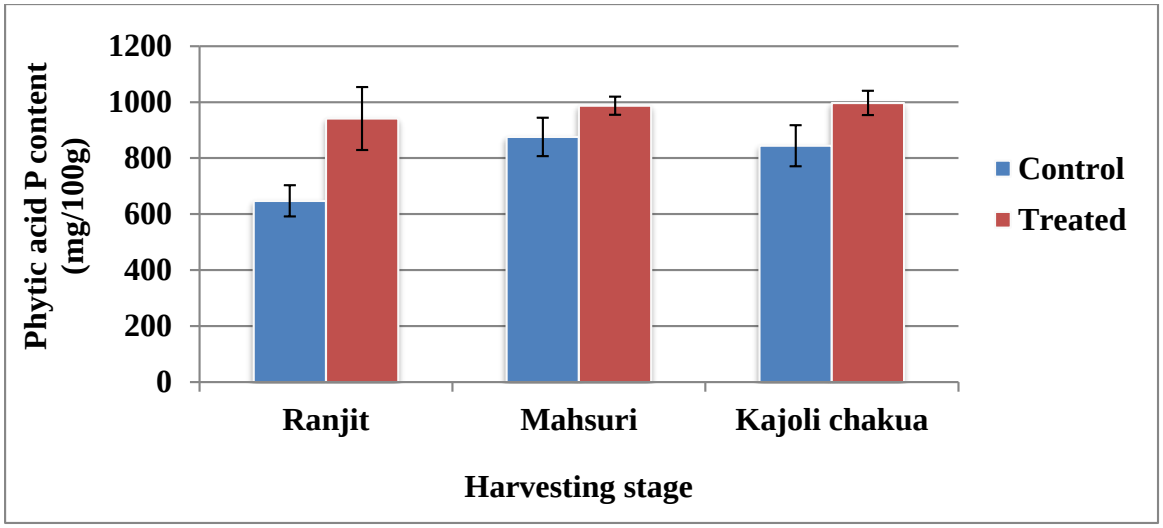


FIG.4.8.1. PHYTIC ACID P CONTENT (mg/100 g, DRY WEIGHT BASIS) OF BROWN RICE AT HARVESTING STAGE

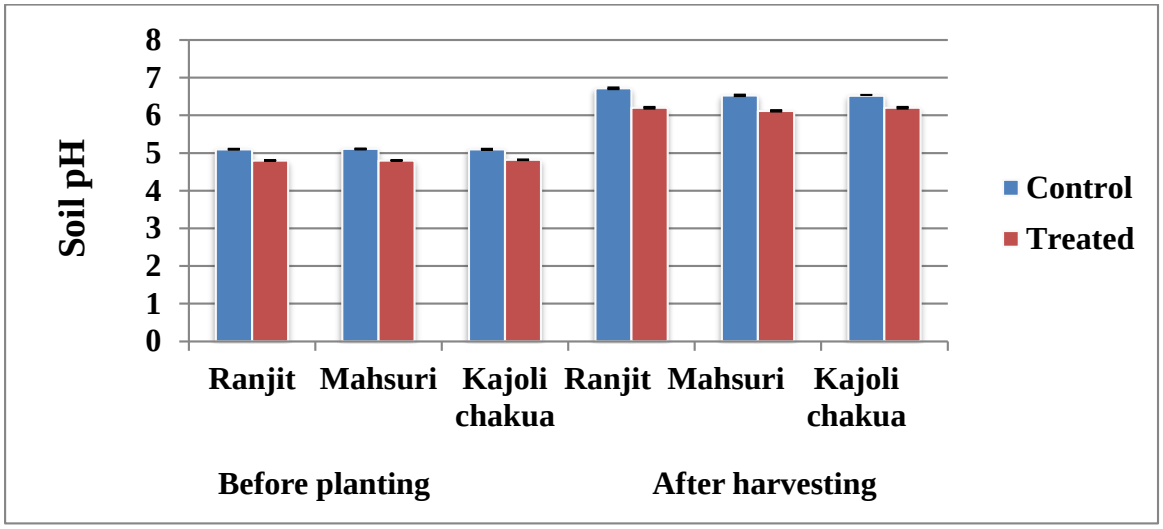


FIG.4.9.1. SOIL pH BEFORE PLANTING AND AFTER HARVESTING

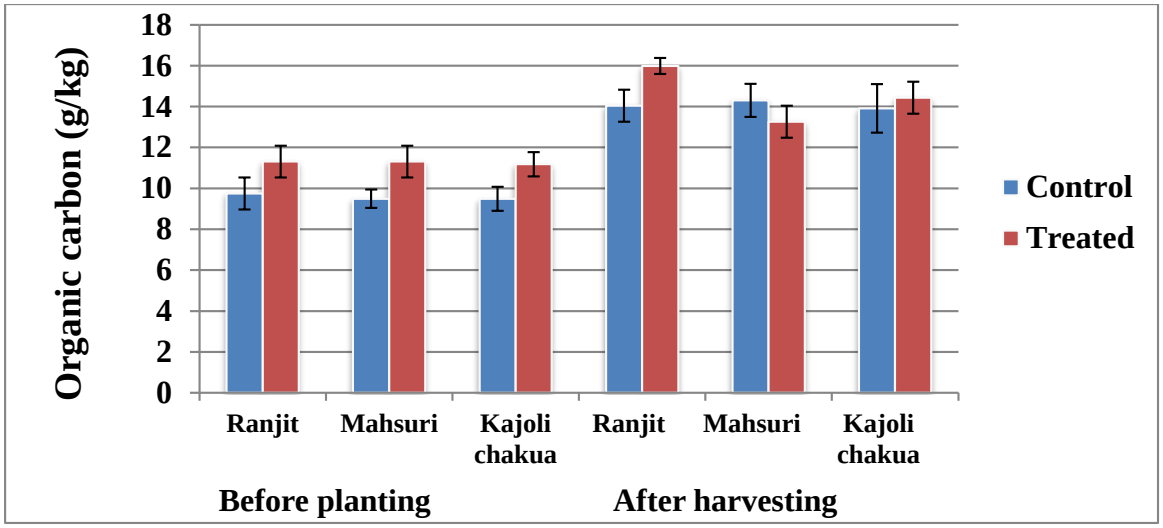


FIG.4.10.1. ORGANIC CARBON (g/kg) OF SOIL BEFORE PLANTING AND AFTER HARVESTING

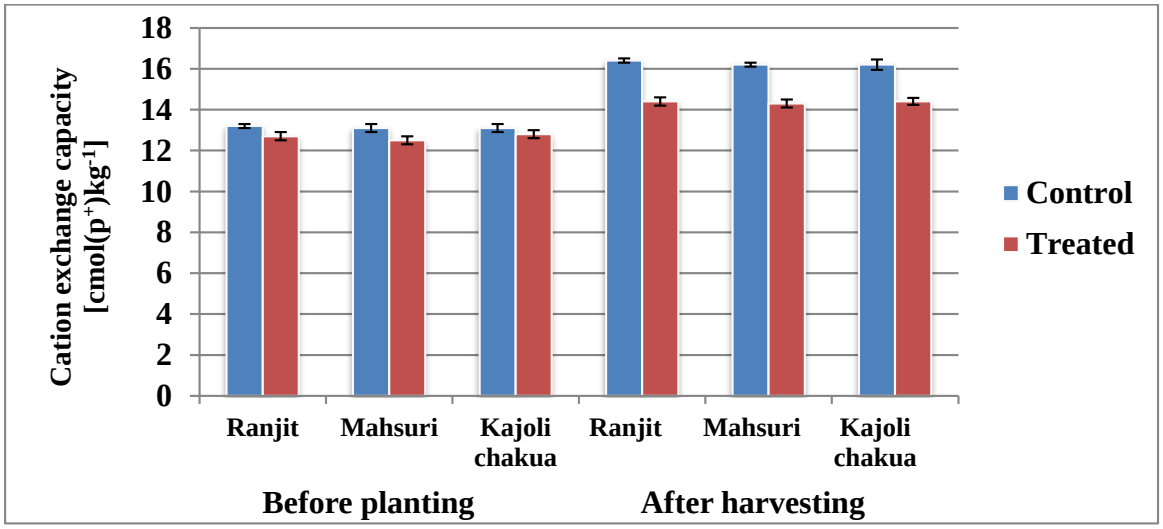


FIG.4.11.1. CATION EXCHANGE CAPACITY [cmol $(p^+)kg^{-1}$] OF SOIL BEFORE PLANTING AND AFTER HARVESTING

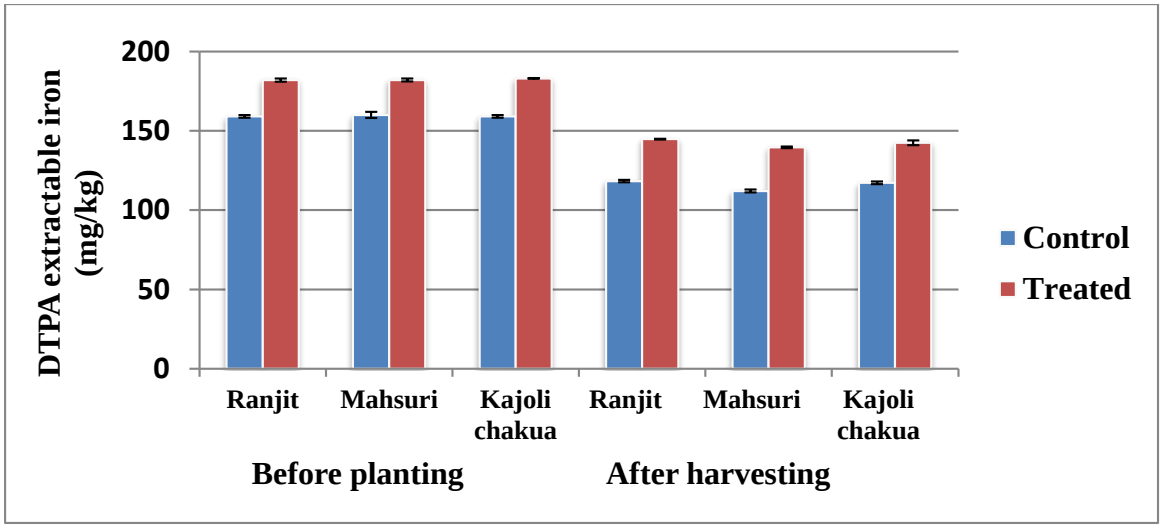


FIG.4.12.1. DTPA EXTRACTABLE IRON (mg/kg) OF SOIL BEFORE PLANTING AND AFTER HARVESTING

CHAPTER V

DISCUSSION

The results obtained in the present study **"Studies on metabolism of iron in rice"** are discussed in this chapter.

5.1. Moisture content

5.1.1. Moisture content of rice leaves

The moisture content is dependent on many factors like variety, yield, application of fertilizer, time of harvesting, etc. The percentage of moisture in a cereal is an important factor for storing, milling and marketing quality of grains (Pathak, 2008).

In the present study, the moisture content on fresh weight basis of rice leaves for three rice varieties at maximum tillering stage ranged from 65.06±0.13% to 75.50±0.20% in control and 67.55±0.38% to 82.36±0.15% in treated plants. At grain filling stage, the moisture content ranged from 71.46±0.01% to 72.97±0.02% in control and 65.87±0.02% to 73.16±0.03% in treated plants. At harvesting stage, the moisture content ranged from 7.26±0.07% to 9.83±0.02% in control and 7.15±0.01% to 10.01±0.10% in treated plants. However, the observed variation among the control and treated plants were non significant.

The present findings of moisture content of rice leaves were found to be comparable with those of Tang *et al*., (2020) which ranged from 26.39% to 60.71%.

5.1.2. Moisture content of paddy and brown rice

In the present study, the moisture content of paddy and brown rice on fresh weight basis for three rice varieties at both grain filling and at harvesting stage varied non significantly.

The moisture content of paddy ranged from 12.93±0.11% to 13.76±0.40 % in control and 11.33±0.30% to 13.60±0.20% in treated plants at grain filling stage. At harvesting stage, the same ranged from 12.73±0.23% to 13.93±0.05% in control and 12.06±0.11% to 14.06±0.23% in treated plants.

The moisture content of brown rice on fresh weight basis ranged from 11.24±0.04% to 12.29±0.05% in control and 11.28±0.04% to 11.98±0.06% in treated plants at grain filling stage. At harvesting stage, the same ranged from 10.11±0.06% to 11.61±0.04% in control and 10.43±0.09% to 11.44±0.04% in treated plants. However, the observed variation among the control and treated plants were non significant.

The present findings of moisture content of paddy was found to be comparable with those of Pominski *et al*., (1961) which ranged from 10% to 14% and brown rice was found to be comparable with those of Oko and Ugwu, (2011), Mbatchou and Dawda, (2013), Dutta *et al*., (2015), Das *et al*., (2018) and Chatterjee and Das, (2019) which were 3.67% to 18.00%, 8.50% to 22.00 %, 12.9 to 13.2%, 10.1% to 11.8 and 12.25% to 15.50%, respectively.

5.2. Total Chlorophyll content

In the present study, the total chlorophyll content (on fresh weight basis) of rice leaves for three rice varieties at maximum tillering stage ranged from 1.17±0.02 mg/g to 1.28±0.02 mg/g in control and 0.88±0.02 mg/g to 1.01±0.02 mg/g in treated plants. At grain filling stage, the same ranged from 2.07±0.02 mg/g to 2.33±0.03 mg/g in control and 1.73±0.03 mg/g to 1.93±0.2 mg/g in treated plants. At harvesting stage, the chlorophyll content ranged from 0.38±0.01 mg/g to 0.57±0.01 mg/g in control and 0.29±0.02 mg/g to 0.49±0.04 mg/g in treated plants.

The present findings of chlorophyll content of rice leaves was found to be comparable with those of Baruah and Nath, (1996) who studied the variation of chlorophyll content on rice leaves due to application of different levels of iron at growth medium. They reported that the chlorophyll content ranged from 2.22 to 2.87 mg/g, 1.52 to 1.92 mg/g and 1.12 to 1.77 mg/g in control (2 ppm), 100 ppm and 200 ppm , respectively. The presents findings was also found to be comparable with those of Saikia and Bhuyan, (2017) who studied the same at different growth stages. They reported that the same ranged from 1.9 to 3.7 mg/g, 2.3to 3.0 mg/g, 2.8 mg/g to 3.0 mg/g and 2.2 to 2.4 mg/g at maximum tillering stage, 2.2 to 3.8 mg/g, 2.8 to 4.8 mg/g, 2.2 mg/g to 4.5 mg/g and 1.3 to 4.4 mg/g at grain filling stage in control, 100 ppm, 200 ppm and 300 ppm iron concentrations of soil, respectively.

The total chlorophyll content was detected to be the highest at grain filling stage followed by maximum tillering stage and harvesting stage, respectively. The observed increase in chlorophyll content at grain filling stage than maximum tillering stage might be due to the distribution of chlorophyll molecules in the leaves at maximum tillering stage leading to decrease in concentration, not the actual content. However, the detection of the lowest chlorophyll content at harvesting stage might be due to degradation of chlorophyll in aged rice plants.

Saikia and Bhuyan, (2017) observed maximum chlorophyll content in plants grown in soils (control) than the soil where iron was treated. They observed that the rice variety *Mahsuri* recorded relatively higher chlorophyll content irrespective of the treatments compared to *Ranjit* and suggested that *Mahsuri* might have been able to maintain higher chlorophyll content through chloroplast development. They suggested that the observed variation of total chlorophyll content at different growth stages, particularly low chlorophyll content at early growing stages might be due to Fe^{2+} mediated ROS which rapidly reduced the chlorophyll content in the rice varieties grown in high iron concentration. However, detection of higher chlorophyll content in *Ranjit* than *Mahsuri* in the present observation might be attributed to lower soil iron content under treated conditions.

In the present study too, it was observed that the chlorophyll content varied significantly at all the three stages analysed and it was higher in plants grown under controlled condition than that of treated. It was also observed that the highest total chlorophyll content was found in *Kajoli chakua* followed by *Ranjit* and *Mahsuri*, respectively.

Baruah and Nath, (1996) also reported lower chlorophyll content of the rice leaves of six rice varieties due to increase in iron content of the growth media and they suggested that higher iron in growth medium affected the total leaf chlorophyll content in iron sensitive varieties. Rout and Sahoo, (2015) referred to the study of Monteiro and Winterbourn (1988) who reported that excess iron amounts must have catalyzed the generation of active O_2 free radical species, which might have eventually oxidized the chlorophyll and subsequently led to decreased chlorophyll content.

It should be noted that the iron atom is required for synthesis of chlorophyll molecules. Chlorophyll is formed in higher plants from the precursor δ-amino-levulinic acid (ALA). This intermediate, ALA, functions for both heme and chlorophyll formation and may be formed through a primary biosynthesis pathway in higher plants requiring a 5-carbon substrate, such as ketoglutarate or glutamate. This pathway may be activated by iron, related to aconitase activity and/or the formation of ferredoxin (Fd). Ferredoxin could be necessary to activate the ALA-synthesizing enzyme. With plants that are iron stressed, Fd would be limiting and directly affect chlorophyll biosynthesis (Miller *et al*., 1984).

5.3. Ash content

The amount of ash present in food sample plays an important role as it determines the level of essential minerals (Barooah *et al.*, 2018). Rice ash contains several minerals of nutritional importance which are present in very small amount (Banik, 2017).

5.3.1. Ash content of rice leaves

In the present study, the ash content on dry weight basis of rice leaves for three rice varieties at maximum tillering stage ranged from 12.00±0.91% to 14.50±0.55% in control and 14.84±0.96% to 15.46±0.61% in treated plants. At grain filling stage, the ash content ranged from 14.25±1.65% to 16.10±0.12% in control and 12.29±0.12% to 14.72±0.08% in treated plants. At harvesting stage, the ash content ranged from 17.81±0.20 % to 20.93±0.12% in control and 16.08±0.08% to 19.73±0.46% in treated plants. The ash content did not vary significantly due to application of additional iron to the soil.

Vadiveloo, (2000) reported the ash content of rice leaf blade, leaf sheath and stem to be 18.5%, 19.1% and 14.0%, respectively on dry weight basis.

5.3.2. Ash content of brown rice

In the present study, the ash content of brown rice on dry weight basis for three rice varieties at grain filling stage ranged from 1.70±0.02% to 2.33±0.11% in control and 1.53±0.11% to 1.87±0.12% in treated plants. However, the variation on ash content among control and treated plants was non significant.

At harvesting stage, in top primary rachis the same ranged from 1.76±0.05% to 1.94±0.04% in control and 1.63±0.15% to 1.82±0.02% in treated plants. In top secondary rachis, the same ranged from 1.70±0.10% to 2.00±0.40% in control and 1.63±0.23% to 1.84±0.04% in treated plants. In middle primary rachis, the ash content ranged from 1.61±0.02% to 1.97±0.02% in control and 1.52±0.28% to 1.82±0.02% in treated plants. In middle secondary rachis, the same ranged from 1.83±0.05% to 2.09±0.10% in control and 1.60±0.34% to 1.78±0.06% in treated plants. In bottom primary rachis, the ash content ranged from 1.90±0.10% to 2.40±0.40% in control and 1.68±0.04% to 1.86±0.05 % in treated plants. In bottom secondary rachis, the same ranged from 1.60±0.06% to 1.82±0.04% in control and 1.61±0.02% to 1.96±0.04% in treated plants. However, only for the ash content of top primary rachis varied significantly among control and treated plants and the ash content at this position of panicle was found to be the highest for variety *Ranjit* followed by *Mahsuri* and *Kajoli chakua*.

The present findings of ash content of brown rice was found to be comparable with those of Oko and Ugwu, (2011), Zubair *et al*., (2012), Mbatchou and Dawda, (2013), Pathak, (2015), Das *et al*., (2018) and Mudoi and Das, (2018) which were 0.50% - 2.0%, 1.48% to 1.98%, 0.86% to 2.48 %, 1.00% to 2.00%, 0.66% to 1.52% and 0.73% to 1.85%, respectively.

5.3.3. Ash content of rice husk

In the present study, the ash content of rice husk for three rice varieties at grain filling stage ranged from 12.53±0.22 % to 14.52±0.04 % in control and 10.28±0.06 % to 13.42±0.04 % in treated plants. At harvesting stage, the same ranged from 12.47% to 14.11±0.15 % in control and 11.39±0.22 % to 12.52±0.12 % in treated plants. However, the ash content at grain filling stage varied significantly among the control and treated plants.

The present findings of the ash content of rice husk was found to be comparable with those of Natarajan and Ganapathy, (2009) and Singh *et al*., (2013) which were 17.09% at 9.45 % moisture content and 9.29% at 4.65% moisture content, respectively.

5.4. Iron content

Iron is one of the essential micronutrients and key co-factors for many enzymes, involved in growth and developmental processes. Iron plays vital roles in metabolic functions and it is the intrinsic component of haemoglobin, myoglobin and cytochromes (Mahesh *et al*., 2015). The iron is required in trace amount but higher concentrations can be harmful. All these elements are essential for normal growth and development as they play important role in nerve functioning, sugar metabolism, activity of numerous enzyme and in cardiac function (Abbas *et al.*, 2011).

Though, there are reports of iron toxicity in rice grown in acid soils under submerged condition due to toxic accumulation of iron in the leaves, hardly any reports are found relating soil iron content with that of grain iron content. Kok *et al*., (2018) emphasized that combined application of both NPK fertilizer and iron fertilizer could be a potential approach to increase iron bioavailability in rice through root development, shoot transport and re-localization, which improved the translocation of iron into rice grain.

It is reported (Backer and Asch, 2005) that the iron toxicity in rice appears for a wide range of soil iron content (20-5000 mg/kg). However, in the present study, the soil iron content in both control and treated were observed to be non toxic at the given level of NPK fertilization.

5.4.1. Iron content of rice leaves

In the present study, the iron content of rice leaves for three rice varieties at maximum tillering stage ranged from 16.12±0.20 mg/100g to 27.64±0.06 mg/100g in control and 33.91±0.03 mg/100g to 54.17±0.25 mg/100g in treated plants. At grain filling stage, the iron content ranged from 18.39±0.12 mg/100g to 58.81±0.26 mg/100g in control and 36.76±0.12 mg/100g to 75.01±0.26 mg/100g in treated in plants. At before harvesting stage, the iron content ranged from 24.77±0.21 mg/100g to 57.58±0.01 mg/100g in control and 26.75±0.14 mg/100g to 69.71±0.19 mg/100g in treated plants.

The iron content of the leaves was significantly higher in treated than in control plants, only at grain filling stage. The highest leaf iron content was detected at grain filling stage for both control (except for *Mahsuri*) and treated plants for all the three rice varieties. The present observation of higher iron content of the

leaves at harvest than at maximum tillering stage for both control and treated (except for *Mahsuri*) plants reflected that iron uptake was not affected by the soil iron content during the growth period. Earlier, Borah *et al*., (2000) reported that at initial stage of growth, iron uptake was more which decreased in later stage due to retarding uptake under low iron levels. They also reported that iron uptake was not affected under high iron levels of soil resulting in relatively more iron content in plants at harvest than maximum tillering stage.

The present study reveals that the iron content of the leaves to be the highest in variety *Ranjit* followed by *Kajoli chakua* and *Mahsuri*, respectively.

The present findings of iron content of rice leaves was found to be comparable with the earlier reports. Nandi, (1997) reported the iron content of rice plants to be 32.03 mg/100g – 34.77 mg/100g, 31.10 mg/100g – 34.07 mg/100g and 26.62 mg/100g – 29.96 mg/100g at 30 DAT, 60 DAT and at harvest, respectively grown under different levels of Fe^{2+} iron content (100-500 ppm) of soil. Das *et al*., (1997) reported the rice plant (leaves) iron content to be 629.8 ppm (62.98 mg/100g) in clay soil to 665.6 ppm (66.56 mg/100g) in sandy loam soil and 597.8 ppm (59.78 mg/100g) in clay soil to 633.3 ppm (63.33 mg/100g) in sandy loam soil at maximum tillering stage and at harvest, respectively. They also observed that the iron levels in the irrigation water above 50 ppm significantly increased the iron concentration in the plants at both maximum tillering stage and at harvest irrespective of soil, having a low and high initial iron content. They also observed that the iron content of plants significantly decreased in the heavy textured soil and iron toxicity symptoms increased progressively in the irrigation water. The rice plant exhibited their tolerance to iron concentrations in the irrigation water only up to 50 ppm in all the soils except in light textured soil having high initial iron content.

The present study revealed that there is increase in leaf iron content with soil iron content. The present finding of detection of higher iron content of leaves for all the varieties grown in treated pots with high soil iron content is also supported by the earlier findings of Das *et al*., (1997) and Borah *et al*.,(2000).

Das *et al*., (1997) reported that the iron content of rice leaves (variety *Mahsuri*) ranged from 93.3 to 1014.7 mg/kg at maximum tillering stage and 84.6 to 914.0 mg/kg at harvest, respectively in soils of low initial iron content (DTPA-Fe

112.5 ppm), which was also irrigated with iron containing water in the range of 0 to 300 ppm. The same at the respective stages ranged from 176.2 to 1018.3 mg/kg and 132.4 to 980.5 mg/kg, respectively in soils of high initial iron content (DTPA-Fe 163.5 ppm), irrigated with iron containing water in the range of 0 to 300 ppm. However, iron toxicity symptoms in the range of mild to moderate was detected for all the three types of soils (sandy clay, silty clay and clay) with high initial iron content, even no iron was applied through irrigation. Increasing the iron content in irrigated water from 50 to 300 ppm lead to mild to extremely severe iron toxicity symptoms.

Borah *et al*., (2000) also reported that the iron content of rice leaves (variety *Mahsuri*) ranged from 61 - 82 mg/kg to 308 - 315 mg/kg at maximum tillering stage, grown in soils of low initial iron content (DTPA-Fe 109 ppm), which was also irrigated with water containing 0 to 50 ppm iron, respectively. The same ranged from 158 - 165 mg/kg to 334 - 342 mg/kg at the same stage, grown in soils of high initial iron content (DTPA-Fe 164 ppm), irrigated with water containing 0 to 50 ppm iron, respectively. At harvest, the same ranged from 52 - 73 mg/kg to 253 - 269 mg/kg, grown in soils of low initial iron content (DTPA-Fe 109 ppm), irrigated with water containing 0 to 50 ppm iron, respectively. However, the iron content ranged from 104 - 112 mg/kg to 248 - 260 mg/kg at the same stage, grown in soils of high initial iron content (DTPA-Fe 164 ppm), irrigated with water containing 0 to 50 ppm iron, respectively. The application of irrigated water up to 50 ppm iron concentration did not produce visual symptoms of toxicity.

In the present finding too, it was observed that within the range of initial soil iron content (DTPA-Fe 159.40 mg/kg to 182.35 mg/kg), no visual symptoms of toxicity developed on the leaves up to 750.13 ppm iron content in the variety *Ranjit*. Borah *et al*., (2000) also did not observe any visual iron toxicity symptoms in rice variety *Mahsuri* up to leaf iron concentration of 408 ppm and 353 ppm at maximum tillering stage and at harvest, respectively; both for sandy clay loam soil and iron containing irrigated water (50 ppm). However, certain reports stated that toxicity symptoms in the leaves appeared at leaf iron content more than 132.4 ppm for the variety *Mahsuri* at harvest (Das *et al*., 1997). Baruah and Bharali, (2015) also stated that there exists large inconsistency within the published literature on iron toxicity with regard to soil iron content (20 to 5000 ppm) and leaf iron

concentration (300 to 2000 ppm). In the present study, the active forms of iron containing antioxidative enzymes (POD, SOD and CAT) might have played some defensive role against development of iron toxicity symptoms at the detected higher level of leaf iron concentration, which has been discussed later on in this chapter. Ravet *et al*., (2009) reported that iron containing protein phytoferritins function as frontline defence mechanism against free iron induced oxidative stress, whose major function is to scavage free reactive iron and prevent oxidative damage. A resistant variety may accumulate more amount of phytoferritin which form complex with iron, reducing iron toxicity damage. Therefore, analysis of phytoferritin in leaves may reveal role of this protein in development of iron toxicity symptoms in rice.

5.4.2. Iron content of brown rice

In the present study, it was observed that the iron content of brown rice for three rice varieties at grain filling stage ranged from 1.17±0.04 mg/100g to 2.18±0.08 mg/100g in control and 4.18±0.03 mg/100g to 5.92±0.10 mg/100g in treated plants.

The iron content of brown rice for three rice varieties at harvesting stage in TPR ranged from 3.26±0.09 mg/100g to 5.18±0.15 mg/100g in control and 7.94±0.01 mg/100g to 11.09±0.22 mg/100g in treated plants. In TSR, the iron content ranged from 3.04 mg/100g to 4.85±0.08 mg/100g in control and 6.89±0.07 mg/100g to 10.62 mg/100g in treated plants. In MPR, the iron content ranged from 2.71±0.04 mg/100g to 4.77 ±0.15 mg/100g in control and 6.77±0.11 mg/100g to 10.54±0.26 mg/100g in treated plants. In MSR, the iron content ranged from 2.28±0.11 mg/100g to 4.04±0.08 mg/100g in control and 6.73±0.06 mg/100g to 10.26±0.36 mg/100g in treated plants. In BPR, the iron content ranged from 1.97±0.13 mg/100g to 3.91±0.11 mg/100g in control and 6.01±0.07 mg/100g to 10.21±0.32 mg/100g in treated plants. In BSR, the iron content ranged from 1.86±0.05 mg/100g to 3.76±0.11 mg/100g in control and 5.88±0.06 mg/100g to 9.67±0.17 mg/100g in treated plants.

The present findings of iron content of brown rice was found to be comparable with the findings of Su *et al*., (2014) which were (0.968 mg/100g to 1.193 mg/100g, 0.926 mg/100g to 1.142 mg/100g, 0.914 mg/100g to 1.133 mg/100g, 0.881 mg/100g to 1.092 mg/100g, 0.840 mg/100g to 1.042 mg/100g and 0.818

mg/100g to 1.008 mg/100g in TPR, TSR, MPR, MSR, BPR and BSR, respectively. Su *et al*., (2014) reported that grain position was an important source of variation for grain mineral concentrations and for all the eight minerals including iron, there were significant differences among grains located on different positions within a panicle. The grains on the top primary and middle primary rachis had substantially higher mineral concentrations than those on the bottom secondary and middle secondary rachis. The present study also indicated variation in grain iron content according to its position on the rachis; the highest being detected at TPR followed by TSR, MPR, MSR, BPR and BSR, respectively.

The present findings of iron content of brown rice was also found to be comparable with the findings of Gregorio *et al*., (2000), Kennedy and Burlingame, (2003), Meng *et al*., (2005), Deepa *et al*., (2008), Sharma *et al*., (2012), Zubair *et al*., (2012) Thongbam *et al*., (2012), Shahid *et al*., (2014), Pathak, (2015b), Kumar *et al*., (2017), Das *et al*., (2018), Chatterjee and Das, (2019) and Mudoi and Das, (2019) which were 1.18 mg/100g to 2.24 mg/100g, 1.01 mg/100g to 2.64 mg/100g, 0.2 mg/100g – 5.2 mg/100g, 1.93 mg/100g to 3.95 mg/100g, 0.029 mg/100g to 0.063 mg/100g, 18.6 mg/100g – 30.1 mg/100g , 2.32 mg/100g to 15.42 mg/100g, 10.1mg/100g to 12.4 mg/100g , 2.38 mg/100g to 4.20 mg/100g, 1.24 mg/100g to 2.41 mg/100g, 1.04 mg/100g to 643.50 mg/100g, 1.54 mg/100g to 4.68 mg/100g and 2.12 mg/100g to 54.40 mg/100g, respectively.

Among the three rice varieties, the highest iron content of brown rice was observed in *Ranjit* followed by *Kajoli chakua* and *Mahsuri* at both the stages. At both the stages, it was found that iron content of brown rice of all the three varieties increased significantly (more than 100 % than that of control) due to added iron content of soil, which raised the DTPA extractable iron to 182.35 mg/kg from 159.40 mg/kg in control. The highest iron content in brown rice was detected in *Ranjit* (10.39±0.47 mg/100g). However, Kok *et al*., (2018) reported that if 15 μg/g iron (1.5 mg/100g, dry weight) was incorporated in polished rice through biofortification, the recommended target was met. Considering maximum 80% loss of iron due to polishing, it can be estimated that the polished form of *Ranjit*, *Mahsuri* and *Kajoli chakua* may retain about 2 mg/100g, 1.34 mg/100g and 1.59 mg/100g iron (dry weight basis), respectively. The observed higher iron content of brown rice

in the present study might also be attributed to NPK fertilization together with iron fertilization.

It has been demonstrated that iron content in rice grain vary according to genotype, geographical conditions and other environmental factors (Anandan *et al*., 2011). Some studies showed that grain position within a panicle had a considerably impact on grain chemical position suggesting that the improvement of spikelet architecture and morphological pattern (particularly for the compact rice genotypes with high grain density and yield levels) is possible for obtaining high yield and balanced nutrition (Calderini and Ortiz-Monasterio, 2003). Large variation among rice grain in a panicle is unfavourable for commercial value and quality (Jeng *et al*., 2006). However, little research has been done on the positional variation of micronutrient concentration in relation of rice panicle morphology (Su *et al*., 2014).

The most important way to improve the iron content in rice is absorption, transport and accumulation of iron and the use of iron fertilizer is one of the agricultural methods. **(**Ying, 2000). However, there is hardly any reference on research work relating soil iron level with that of grain iron level. As in South-East Asia, rice cultivation in waterlogged acid soil leads to iron toxicity for some of the susceptible varieties leading to 30-60% loss in yield (Sahrawat 2000 and Majerus *et al*., 2007), there is scope for increasing the grain iron content of rice by cultivating tolerant rice varieties in those areas and also by adding additional iron through irrigated water in soils of low initial iron content. Das *et al*., (1997) and Borah *et al*., (2000) already reported that soil types had influence on plant (leaves) iron content at both maximum tillering stage and at harvest and they observed positive influence of the soil clay content towards reducing the iron concentration in the plant (leaves).

Although, much attention has been paid to the role of brown rice as nutrition source, it cannot become the daily food (Domene *et al*., 2001). However, utilisation of brown rice to various rice products such as flaked rice, popped rice and puffed rice may lead to improvement of iron nutrition among rice eating population of our country.

5.4.3. Iron content of rice husk

In the present study, it was observed that the iron content of rice husk for three rice varieties at grain filling stage ranged from 3.11±0.02 mg/100g to

5.50±0.30 mg/100g in control and 6.96±0.10 mg/100g to 9.37±0.01 mg/100g in treated plants. At harvesting stage, it was observed that the iron content of rice husk of three rice varieties ranged from 6.95±0.18 mg/100g to 8.91±0.01 mg/100g in control and 11.17±0.32 mg/100g to 12.07±0.30 mg/100g in treated plants.

The present findings of iron content of rice husk was found to be comparable with the findings of Meng *et al*., (2005) which were 3.9 to 9.5 mg/100 g fresh weight (on 14 % moisture level).

In the present study, the iron content of rice husk was found to be significantly higher in treated than in control. At both the stages, the iron content of rice husk for the three varieties were higher than the brown rice. However, the iron content of the rice husk was higher at harvesting stage than at grain filling stage, which indicated transport and accumulation of iron till the maturity of the grain. The highest iron content of rice husk was found for the variety *Ranjit* followed by *Kajoli chakua* and *Mahsuri*, respectively.

5.5. Peroxidase (POD) activity

5.5.1. Total activity of peroxidase extract prepared from rice leaves

In the present study, the total activity of peroxidase extract prepared from rice leaves for three rice varieties at maximum tillering stage ranged from 21.14±0.04 to 22.81±0.01 units/min/g fresh weight in control and 21.86±0.11 to 23.12±0.07 units/min/g fresh weight in treated plants. At grain filling stage, the total activity ranged from 21.74±0.03 to 22.80±0.05 units/min/g fresh weight in control and 21.96±0.02 to 23.00±0.20 units/min/g fresh weight in treated plants. At harvesting stage, the total activity of peroxidase ranged from 22.64±0.04 to 24.02±0.03 units/min/g fresh weight in control and 22.82±0.03 to 24.14±0.04 units/min/g fresh weight in treated plants.

In the present study, the total activity of peroxidase for three rice varieties was found to be significantly higher in treated than in control only at maximum tillering stage.

In almost all the three stages, the highest total activity of peroxidase was found in *Mahsuri* followed by *Kajoli chakua* and *Ranjit*, respectively.

5.5.2. Total activity of peroxidase extract prepared from brown rice

In the present study, the total activity of peroxidase extract prepared from brown rice for three rice varieties at grain filling stage ranged from 19.33±0.31 to 20.06±0.4 units/min/g fresh weight in control and 20.26±0.24 to 20.86±0.22 units/min/g fresh weight in treated plants. At harvesting stage, the total activity of peroxidase ranged from 21.02±0.42 to 21.79±0.33 units/min/g fresh weight in control and 21.65±0.61 to 22.10±0.14 units/min/g fresh weight in treated plants. At grain filling stage, the total activity of peroxidase of brown rice was significantly higher in treated plants than in control. Total activity of peroxidase of respective varieties increased at harvesting stage than at grain filling stage.

In the present study, it was found that the total activity of peroxidase was less in brown rice than in leaves. The higher iron content, detected in the leaves than in the brown rice might be the constituent of peroxidase which led to higher activity in leaves.

In both the stages, the lowest total activity of peroxidase was recorded in *Ranjit* and the highest in *Mahsuri* for treated and *Kajoli chakua* for control.

5.5.3. Specific activity of peroxidase extract prepared from rice leaves

In the present study, the specific activity of peroxidase extract prepared from rice leaves for three rice varieties at maximum tillering stage ranged from 48.47±1.36 to 50.34±1.10 units/mg protein in control and 57.80±1.36 to 78.09±2.63 units/mg protein in treated. At grain filling stage, the specific activity ranged from 49.92±2.20 to 54.28±1.41 units/mg protein in control and 55.55±1.37 to 60.52±1.31 units/mg protein in treated. At harvesting stage, the specific activity of peroxidase ranged from 36.33±1.24 to 42.15±1.13 units/mg protein in control and 43.88±1.10 to 55.49±1.19 units/mg protein in treated.

The specific activity of peroxidase extract prepared from leaves for three rice varieties was found to be non significant at all the stages.

Rossatto, *et al*., (2017) reported the specific activity of peroxidase in leaves of rice variety “BRS AG” (cultivated for ethanol production and animal feed) in both control and salt (NaCl) stressed condition (136 mM). At 10 days after germination, the specific activity of peroxidase was found to be higher (2.4 μmol/min/mg protein) in stressed condition than in control (1.2 μmol/min/mg

protein). However, at 15 and 20 days after germination, the same was observed to be higher in control than in stressed condition.

Poli *et al*., (2018) observed higher specific activity of antioxidative enzymes (SOD, POD and CAT) in rice varieties grown in soil of low P content (stressed condition) than in soils containing normal levels of P. They analysed the rice leaves both at vegetative stage and reproductive stage.

5.5.4. Specific activity of peroxidase extract prepared from brown rice

In the present study, the specific activity of peroxidase extract prepared from brown rice for three rice varieties at grain filling stage ranged from 2.50±0.01 to 2.99±0.01 units/mg protein in control and 2.89±0.03 to 3.61±0.05 units/mg protein in treated plants. At harvesting stage, the specific activity of peroxidase ranged from 1.90±0.01 to 2.21±0.02 units/mg protein in control and 2.03±0.02 to 3.84±0.04 units/mg protein in treated plants. It was significantly higher in treated plants at grain filling stage only.

The total activity of peroxidase increased at harvesting stage than grain filling stage. At grain filling stage, the highest specific activity of peroxidase was found in *Ranjit* for treated and *Kajoli chakua* for control and for both, the least was detected in *Mahsuri*, respectively.

In the present study, it was found that both the total activity and specific activity were higher in leaves at both the stages, grain filling and harvesting. This might be due to detection of comparatively higher amount of iron in leaves, together with lower protein content of the leaves. The amount of active peroxidase per mg soluble protein is higher in leaves than in brown rice.

5.6. Superoxide dismutase activity

5.6.1. Total activity of superoxide dismutase extract prepared from rice leaves

In the present study, the total activity of superoxide dismutase extract prepared from rice leaves for three rice varieties at maximum tillering stage ranged from 0.50±0.01 to 0.75±0.05 units/min/g fresh weight in control and 0.75±0.03 to 1.25±0.01 units/min/g fresh weight in treated plants. At grain filling stage, the total activity ranged from 0.50±0.02 to 1.25±0.03 units/min/g fresh weight in control and 1.00±0.05 to 1.50±0.03 units/min/g fresh weight in treated plants. At harvesting

stage, the total activity ranged from 0.25±0.01 to 1.25±0.02 units/min/g fresh weight in control and 0.50±0.03 to 1.50±0.01 units/min/g fresh weight in treated plants.

The total activity of superoxide dismutase for three rice varieties were found to be higher in grain filling stage followed by harvesting stage and maximum tillering stage.

The total activity of superoxide dismutase for three rice varieties was found to be significantly higher in treated than in control in all the three stages. The highest total activity of superoxide dismutase was found in *Ranjit* followed by *Mahsuri* and *Kajoli chakua* at maximum tillering stage. In the later two stages, the highest total activity of superoxide dismutase was found in *Ranjit* followed by *Kajoli chakua* and *Mahsuri*.

The SOD activity in plant not only depends on the production of ROS, it also depends on the type of rice varieties, nature of stress, degree and duration of stress (Saikia and Baruah, 2012). During active tillering stage, higher total SOD activity was detected by Saikia and Baruah, (2012) in variety *Ranjit* than *Mahsuri* in controlled soil. However, with increase in Fe^{2+} concentration, the variety *Ranjit* recorded a sharp decrease in SOD activity which might be due to enzyme inhibition by the higher iron levels. They detected that the variety *Mahsuri* recorded an increasing trend of SOD activity at higher iron concentration of soil, which might be due to limited Fe^{2+} uptake and active oxygen detoxifying mechanism of this rice variety.

However, in the present investigation, the total SOD activity was found to be higher for all the varieties grown in higher iron content of soil. The observed differences of present investigation with that of Saikia and Baruah, (2012) might be due to growing of plants in much higher concentration of Fe^{2+} (100 ppm to 300 ppm Fe^{2+}) by the later who already reported that the activity of SOD depends on the degree of stress.

5.6.2. Total activity of superoxide dismutase extract prepared from brown rice

In the present study, the total activity of superoxide dismutase extract prepared from brown rice for three rice varieties at grain filling stage ranged from 0.50±0.01 to 1.50±0.02 units/min/g fresh weight in control and 1.00±0.05 to 1.50±0.01 units/min/g fresh weight in treated plants. At harvesting stage, the total

activity ranged from 0.25±0.03 to 1.00±0.02 units/mg protein in control and 1.25±0.02 to 1.50±0.03 units/min/g fresh weight in treated plants. The variation in the total activity of SOD in brown rice was found to be non significant regarding different levels of soil iron content.

5.6.3. Specific activity of superoxide dismutase extract prepared from rice leaves

In the present study, the specific activity of superoxide dismutase extract prepared from rice leaves for three rice varieties at maximum tillering stage ranged from 1.13±0.20 to 2.34±0.22 units/mg protein in control and 1.59±0.22 to 2.60±0.31 units/mg protein in treated plants. At grain filling stage, the specific activity ranged from 1.85±0.21 to 3.12±0.20 units/mg protein in control and 2.63±0.30 to 4.05±0.32 units/mg protein in treated plants. At harvesting stage, the specific activity of superoxide dismutase ranged from 0.55±0.22 to 2.45±0.21 units/mg protein in control and 1.04±0.21 to 4.05±0.32 units/mg protein in treated plants.

In the present study, the specific activity of superoxide dismutase for three rice varieties was found to be significantly higher in treated than in control only at grain filling stage. At grain filling stage, the highest specific activity of superoxide dismutase was found to be the highest in *Ranjit* followed by *Kajoli chakua* and *Mahsuri* for treated, whereas in control, the same was found to be the highest for *Kajoli chakua* followed by *Ranjit* and *Mahsuri.*

Rossatto, *et al*., (2017) reported the specific activity of SOD in leaves of rice variety “BRS AG” (cultivated for ethanol production and animal feed) in both control and salt (NaCl) stressed condition (136 mM). At 10 and 15 days after germination, the specific activity of SOD was found to be higher in stressed condition than in control. However, at 20 days after germination, the same was observed to be higher in control than in stressed condition.

5.6.4. Specific activity of superoxide dismutase extract prepared from brown rice

In the present study, the specific activity of superoxide dismutase extract prepared from brown rice for three rice varieties at grain filling stage ranged from 0.57±0.03 to 1.72±0.01 units/mg protein in control and 1.27±0.03 to 1.97±0.02

units/mg protein in treated plants. At harvesting stage, the specific activity of superoxide dismutase ranged from 0.25±0.01 to 0.98±0.02 units/mg protein in control and 1.28±0.02 to 2.20±0.03 units/mg protein in treated plants.

At both the stages, the specific activity of SOD was found to be non significant irrespective of level of iron content of soil.

Detection of higher level of specific activity of SOD in leaves than in brown rice at both grain filling and harvesting stages indicated higher amount of active SOD per mg protein of the superoxide dismutase extract in leaves.

5.7. Catalase activity

Catalase activities may play a role in iron tolerance in rice (Bode *et al*., 1995).

5.7.1. Total activity of catalase extract prepared from rice leaves

In the present study, the total activity of catalase extract prepared from rice leaves for three rice varieties at maximum tillering stage ranged from 79.50±0.69 to 109.37±0.60 units/min/g fresh weight in control and 92.62±0.32 to 122.06±1.38 units/min/g fresh weight in treated plants. At grain filling stage, the total activity ranged from 83.43±0.22 to 107.87±0.52 units/min/g fresh weight in control and 80.50±0.09 to 114.50±0.48 units/min/g fresh weight in treated plants. At harvesting stage, the total activity ranged from 74.18±0.14 to 80.37±0.44 units/min/g fresh weight in control and 76.37±0.18 to 85.18±0.53 units/min/g fresh weight in treated plants.

The total activity of catalase from leaves for three rice varieties was found to be significantly higher in treated than in control only at maximum tillering stage. At maximum tillering stage, the highest total activity of catalase was found in *Kajoli chakua* followed by *Ranjit* and *Mahsuri*. Saikia and Baruah, (2012) also observed higher total activity of catalase in *Ranjit* than in *Mahsuri* at active tillering stage at 100 ppm Fe^{2+} concentration of soil, which later on reversed with further increase of soil iron content.

The total activity was found to decrease with the development of the plant, being the highest at maximum tillering stage and the least at harvesting stage.

5.7.2. Total activity of catalase extract prepared from brown rice

In the present study, the total activity of catalase extract prepared from brown rice for three rice varieties at grain filling stage ranged from 122.81±0.21 to 127.75±0.79 units/min/g fresh weight in control and 128.12±0.40 to 128.93±0.43 units/min/g fresh weight in treated plants. At harvesting stage, the total activity ranged from 90.87±0.05 to 104.31±0.26 units/mg protein in control and 98.25±0.34 to 119.00±0.10 units/min/g fresh weight in treated plants.

The variation in the total activity of catalase of brown rice was found to be non significant regarding different levels of soil iron content. At both grain filling and harvesting stage, the total activity of catalase was observed to be higher in brown rice than in the leaves, which might be due to lower moisture content of brown rice than the leaves particularly at grain filling stage and the presence of more amount of active catalase in brown rice than in leaves. Although, higher iron content was detected in the leaves than in the brown rice, most of the iron atoms in leaves might be present in other iron containing molecules than in catalase.

5.7.3. Specific activity of catalase extract prepared from rice leaves

In the present study, the specific activity of catalase extract prepared from rice leaves for three rice varieties at maximum tillering stage ranged from 53.71±0.29 to 71.62±0.32 units/mg protein in control and 59.75±0.26 to 80.16±0.40 units/mg protein in treated plants. At grain filling stage, the specific activity ranged from 56.00±0.28 to 72.88±0.22 units/mg protein in control and 57.91±0.30 to 86.74±0.31 units/mg protein in treated plants. At harvesting stage, the specific activity of superoxide dismutase ranged from 45.51±0.22 to 50.23±0.25 units/mg protein in control and 45.73±0.26 to 51.02±0.34 units/mg protein in treated plants.

In the present study, the specific activity of catalase for three rice varieties was found to be significantly higher in treated than in control only at maximum tillering stage. At maximum tillering stage, the highest specific activity of catalase was found in *Ranjit* followed by *Kajoli chakua* and *Mahsuri*. As in total activity, the highest specific activity was detected to be the highest at maximum tillering stage followed by grain filling stage and harvesting stage.

Rossatto, *et al*., (2017) reported the specific activity of catalase in leaves of rice variety “BRS AG” (cultivated for ethanol production and animal feed)

in both control and salt (NaCl) stressed condition (136 mM). At 10, 15 and 20 days after germination, the specific activity of catalase in leaves was found to be higher in stressed condition than in control.

5.7.4. Specific activity of catalase extract prepared from brown rice

In the present study, the specific activity of catalase extract prepared from brown rice for three rice varieties at grain filling stage ranged from 7.48±0.09 to 7.73±0.04 units/mg protein in control and 7.64±0.07 to 8.50±0.04 units/mg protein in treated plants. At harvesting stage, the specific activity of catalase ranged from 4.01±0.03 to 4.33±0.03 units/mg protein in control and 4.19±0.02 to 4.76±0.04 units/mg protein in treated plants.

The specific activity of catalase in brown rice at both the stages were found to be non significant irrespective of the iron content of soil. However, the specific activity in brown rice was higher at grain filling stage than at harvesting stage.

Detection of higher level of specific activity of catalase in leaves than in brown rice at both grain filling and harvesting stages indicated higher amount of active catalase per mg protein of the catalase extract in leaves.

Comparison of activity of three antioxidative enzymes

1. The activities of the enzymes analysed showed significant variation regarding soil iron content in most of the growth stages for all the three varieties.

2. It was observed that activities of POD and SOD were higher during later stages of growth, SOD being mostly active in grain filling stage than the other two stages; whereas, activities of CAT was higher at initial stage of growth (maximum tillering followed by grain filling and harvesting stage).

3. The higher activity for CAT and SOD were recorded for variety *Ranjit,* than another improved variety *Mahsuri*; whereas, for POD, activity was found to be higher in *Mahsuri* than *Ranjit*.

4. In all the three cases, the specific activity was higher in leaves than in brown rice.

However, references on antioxidative enzyme activities in brown rice are very limited.

5.8. Phytic acid P content

Phytic acid (PA; myo-inositol hexaphosphate) is a ubiquitous biomolecule present abundantly in plants and PA phosphorus constitutes the major portion of total phosphorus in several seeds and grains. It accounts for 50-80% of the total phosphorus in different cereals. The presence of certain forms of a particular nutrient can hinder the uptake of other nutrients especially micronutrients. Phytic acid forms insoluble complexes with polycations like zinc and iron due to reactive phosphorus groups attached to its inositol ring which, in turn renders these essential nutrients unavailable for human intestinal absorption. Wet processing like soaking, germination and fermentation of food crops reduced phytic acid content and increased the solubility of nutrients (Mahesh *et al*., 2015).

For many years, phytic acid was considered an antinutritional compound because it reduces the bioavailability of several minerals important for human nutrition. Since 1990s, phytic acid has been scientifically emphasized for its beneficial effects on human health, particularly in the prevention of diabetes, Parkinson's disease and cancer (Kumar *et al.*, 2017).

In the present study, the phytic acid P content of brown rice for three rice varieties at harvesting stage ranged from 647.46±55.43 to 875.85±68.41 mg/100g in control and 941.82±112.40 to 997.11±43.26 mg/100g in treated plants.

In the present study, the highest phytic acid P content was found in *Mahsuri* followed by *Kajoli chakua* and *Ranjit* in control and *Kajoli chakua* followed by *Mahsuri* and *Ranjit* in treated, respectively.

The present findings of phytic acid P content of brown rice was found to be comparable with those of Liu *et al*., (2005a), Liu *et al*., (2005b), Wei *et al*., (2007), Magdy *et al*., (2010), Su *et al*., (2014), Bhattacharjee, (2019) which were 685 mg/100g to 1030 mg/100g, 642 mg/100g to 1021 mg/100g, 699 mg/100g to 1034 mg/100g, 411.25 mg/100g to 750.0 mg/100g, 368 mg/100g to 613 mg/100g and 303.46 mg/100g, respectively.

5.9. Soil pH

In the present study, pH of soil before planting ranged from 5.10±0.01 to 5.11±0.01 in control and 4.80±0.01 to 4.82±0.01 in treated soils. After harvesting, the same ranged from 6.52±0.02 to 6.72±0.02 in control and 6.12±0.02 to 6.20±0.02

in treated soils. It was observed that the pH of soil was significantly lower in treated than in control soils, both before planting and after harvesting which might be due to release of H^+ following dissolution of $FeSO_4.7H_2O$ in water.

The soil pH values observed in the present study are comparable with those of Das *et al*., (1997), Borah *et al*., (2000) and Bhuyan *et al*., (2014). Das *et al*., (1997) and Borah *et al*., (2000) reported the pH to be 5.2 and 5.1 for soils of Nagaon district, Assam, for soils with low initial iron content (DTPA-Fe 112.5 mg/kg and DTPA-Fe 109 mg/kg, respectively) and 4.9 for soils of the same district with high initial iron content (DTPA-Fe 163.5 mg/kg and DTPA-Fe 164 mg/kg, respectively). Bhuyan *et al*., (2014) reported the pH to be 4.60 to 6.61 for soils of Lakhimpur district, Assam with DTPA-Fe 36.4mg/kg to 224.0 mg/kg.

5.10. Soil organic carbon

In the present study, the soil organic carbon before planting ranged from 9.49±0.45 g/kg to 9.75±0.78 g/kg in control and 11.18±0.59 g/kg to 11.31±0.78 g/kg in treated soils. After harvesting, the same ranged from 13.91±1.19 g/kg to 14.04±0.78 g/kg in control and 13.26±0.78 g/kg to 15.99±0.39 g/kg in treated soils. It was observed that the organic carbon was significantly higher in treated soils only before planting. However, the increase in soil organic carbon after harvest in both control and treated soils might be due to decomposition of root cells in soil.

The soil organic carbon values observed in the present study are comparable with those of Das *et al*., (1997), Borah *et al*., (2000) and Bhuyan *et al*., (2014) which were 12.30 g/kg for soils with low initial iron content (DTPA-Fe 112.5 mg/kg), 13.40 for soils with high initial iron content (DTPA-Fe 163.5 mg/kg); 10.60 g/kg for soils with low initial iron content (DTPA-Fe 109 mg/kg), 12.60 g/kg for soils with high initial iron content (DTPA-Fe 164 mg/kg) and 1.20 g/kg to 18.30 g/kg (DTPA-Fe 36.4 to 224 mg/kg), respectively. Bhuyan *et al*., (2014) reported increase in DTPA-Fe content with increase in soil organic carbon.

5.11. Cation exchange capacity of soil

In the present study, the cation exchange capacity of soil before planting ranged from 13.10±0.20 to 13.20±0.10 cmol (p^+) kg^{-1} in control and 12.50±0.20 to 12.80±0.20 cmol (p^+) kg^{-1} in treated soils. After harvesting, the same ranged from 16.20±0.10 to 16.40±0.10 cmol (p^+) kg^{-1} in control and 14.30±0.10 to

14.40±0.20 cmol (p^+) kg^{-1} in treated soils. The increase in cation exchange capacity of soil after harvest of crop might be due to the effect of enhanced organic carbon content of soil. The cation exchange capacity significantly differed between control and treated soils at both, before planting and after harvesting which might be due to difference in pH-dependant charge with corresponding change in soil pH.

The cation exchange capacity of soil values observed in the present study are comparable with those of Das *et al*., (1997), Borah *et al*., (2000) and Bhuyan *et al*., (2014) which were 18.6 cmol (p^+) kg^{-1} for soils with low initial iron content (DTPA-Fe 112.5 mg/kg), 18.4 cmol (p^+) kg^{-1} for soils with high initial iron content (DTPA-Fe 163.5 mg/kg); 17.2 cmol (p^+) kg^{-1} for soils with low initial iron content (DTPA-Fe 109 mg/kg), 18.4 cmol (p^+) kg^{-1} for soils with high initial iron content (DTPA-Fe 164 mg/kg) and 3.30 cmol (p^+) kg^{-1} to 14.00 cmol (p^+) kg^{-1} (DTPA-Fe 36.4 to 224 mg/kg), respectively.

5.12. DTPA extractable iron of soil

In the present study, the DTPA extractable iron of soil before planting ranged from 159.10±0.90 to 160.00±2.00 mg/kg in control and 182.01±0.99 to 183.01±0.04 mg/kg in treated soils. After harvesting, the same ranged from 112.08±0.92 to 118.25±0.75 mg/kg in control and 139.53±0.47 to 144.75±0.25 mg/kg in treated soils. It was observed that the DTPA extractable iron was found to be significantly higher in treated than in control soils, both before planting and after harvesting, which might be due to application of iron solution in treated soils. However, the observed differences in DTPA extractable iron content in soils of the control pots and in treated pots after harvest than the same before planting might be due to uptake of DTPA extractable Fe by the plant for its growth. The difference was more for the treated soils than that of control soils. More accumulation of iron in plants grown in treated soil also supports this finding.

The DTPA extractable Fe of soil values observed in the present study are comparable with those of Das *et al*., (1997), Borah *et al*., (2000) and Bhuyan *et al*., (2014) which were 112.5 mg/kg to 163.5 mg/kg, 109 mg/kg to 164 mg/kg and 36.4 mg/kg to 224 mg/kg, respectively for soils of Nagaon and Lakhimpur district of Assam.

CHAPTER VI

CONCLUSION

Based on the results of the present study **"Studies on metabolism of iron in rice",** the following conclusions can be drawn.

1. Application of additional iron to the soil (1L solution of $Fe_2SO_4.7H_2O$ at 50 ppm concentration to 6 kg soil) did not affect significantly on moisture content (in both leaves and brown rice), ash content of leaves, iron content of leaves (both, at maximum tillering and harvesting stage) and phytic acid P content of brown rice.

2. Higher the soil iron content, significantly lower was the chlorophyll content in rice leaves.

3. The ash content was significantly lower in treated plants (both, in TPR of brown rice at harvesting stage and rice husk at grain filling stage) than the same in control.

4. For higher soil iron content (DTPA extractable Fe 182.35 mg/kg), more was the uptake by plants (both leaves and grains) at all the growth stages analysed (maximum tillering stage, grain filling stage and harvesting stage). In brown rice, the uptake of iron was up to more than 100% than that of control.

5. Position of grain in the panicle affected accumulation of iron in brown rice. It was found in the following order : TPR>TSR>MPR>MSR>BPR>BSR.

6. Regarding the varietal difference, the highest uptake of iron was found in the rice variety *Ranjit* followed by *Kajoli chakua* and *Mahsuri.*

7. There was no iron toxicity symptom at the initial DTPA extractable Fe (159.4 mg/kg) and at treated (182.35 mg/kg).

8. Specific activity of the anti-oxidative enzymes in rice leaves were found to be significantly higher in treated plants: for SOD at grain filling stage and CAT at maximum tillering stage, while, specific activity of POD was non significant.

9. Specific activity of the anti-oxidative enzymes in brown rice were found to be significantly higher in treated plants only for POD at grain filling stage and non significant for other two enzymes.

10. Application of additional iron to the soil led to significant decrease in soil pH and cation exchange capacity, but increase in organic carbon and DTPA extractable Fe.

11. Considering the average rice consumption per capita to be 189 g/day (www.statista.com retrived on 22/09/2020) and the daily requirement of iron for people of different age group (from children to adult women) to be 20 mg/day (Nair and Augustine, 2018), it is calculated that in 189 g raw brown rice of these three rice varieties, on average 15.76 mg iron is present which is sufficient to fulfil 78.8 % of daily iron requirement. It is reported that the absorption of iron is on average 5 to 8% in people of different age groups, particularly children to women (Nair and Augustine, 2018).

12. **Future prospects :**

i) Quantitative analysis of ferritin in brown rice may reveal information regarding participation of observed higher level of iron atoms in ferritin molecule formation.

ii) Repetition of such study involving widely available rice germplasms of Assam in soils ranging from sandy clay to clay including application of additional iron, considering initial soil iron status may be beneficial to increase levels of iron content in some brown rice varieties without producing iron toxicity symptoms.

iii) The scope of incorporation of genes for phytase and increasing the cysteine amino acid residues in the proteins of brown rice may be studied to know their effect on increasing the bioavailability of iron from rice grain.

Finally, it may be concluded that the present study clearly suggests that application of additional iron solution (1L) at 50 mg/L concentration to 6 kg of soil having initial DTPA extractable Fe 159.4 mg/kg led to increase in iron content of brown rice (more than 100% in comparison to control) in all the three varieties (*Ranjit, Mahsuri* and *Kajoli chakua*). Therefore, considering initial iron status of the soil, application of iron solution of suitable concentration together with NPK

fertilization may be advocated for increasing grain iron content of these three rice varieties.

Although, much attention has been paid to the role of brown rice as nutrition source, it cannot become the daily food. However, utilisation of brown rice to various rice products such as flaked rice, popped rice, puffed rice, etc. may lead to improvement of iron nutrition among rice eating population of our country.

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*Original not seen

ANNEXURE I

Total soluble protein content (mg/g, fresh weight basis) in peroxidase extract prepared from rice leaves at three different growth stages

Variety/ Stage	Maximum tillering stage		Grain filling stage		Harvesting stage	
	Control	**Treated**	**Control**	**Treated**	**Control**	**Treated**
Ranjit	0.42±0.02	0.28±0.02	0.41±0.01	0.37±0.01	0.59±0.01	0.42±0.01
Mahsuri	0.46±0.01	0.40±0.01	0.45±0.03	0.38±0.01	0.57±0.01	0.52±0.01
Kajoli chakua	0.46±0.01	0.35±0.02	0.42±0.02	0.40±0.01	0.65±0.01	0.54±0.01
Mean	0.44±0.03	0.34±0.06	0.42±0.02	0.38±0.01	0.60±0.05	0.49±0.06
t value		6.04		2.98		2.20
P value		0.02*		0.09 NS		0.15 NS
SE (m)	0.01	0.03	0.01	0.01	0.03	0.03

The data represented are the mean of three replications ± Standard deviation

*Significant at 5% level of probability

NS : Not significant

ANNEXURE II

Total soluble protein content (mg/g, fresh weight basis) in peroxidase extract prepared from brown rice at two different growth stages

Variety/ Stage	Grain filling stage		Harvesting stage	
	Control	Treated	Control	Treated
Ranjit	6.63±0.30	5.60±0.26	9.86±0.11	5.66±0.57
Mahsuri	8.00±0.20	7.20±0.20	11.60±0.26	10.86±0.11
Kajoli chakua	6.70±0.20	6.20±0.10	11.03±0.20	9.30±0.43
Mean	7.11±0.77	6.33±0.80	10.83±0.88	8.60±2.66
t value		5.06		2.16
P value		0.03*		0.16 NS
SE (m)	0.44	0.46	0.51	1.54

The data represented are the mean of three replications ± Standard deviation

*Significant at 5% level of probability

NS : Not significant

ANNEXURE III

Total soluble protein content (mg/g, fresh weight basis) in superoxide dismutase extract prepared from rice leaves at three different growth stages

Variety/ Stage	Maximum tillering stage		Grain filling stage		Harvesting stage	
	Control	**Treated**	**Control**	**Treated**	**Control**	**Treated**
Ranjit	0.32±0.02	0.48±0.01	0.41±0.02	0.37±0.01	0.51±0.01	0.37±0.01
Mahsuri	0.31±0.01	0.40±0.01	0.27±0.02	0.38±0.01	0.45±0.02	0.48±0.01
Kajoli chakua	0.44±0.05	0.47±0.01	0.32±0.02	0.41±0.01	0.40±0.01	0.38±0.01
Mean	0.35±0.07	0.45±0.04	0.33±0.07	0.38±0.02	0.45±0.05	0.41±0.06
t value		-2.48		-1.13		0.85
P value		0.13 NS		0.37 NS		0.48 NS
SE (m)	0.04	0.02	0.04	0.01	0.03	0.03

The data represented are the mean of three replications ± Standard deviation

NS : Not significant

ANNEXURE IV

Total soluble protein content (mg/g, fresh weight basis) of superoxide dismutase extract prepared from brown rice at two different growth stages

Variety/ Stage	Grain filling stage		Harvesting stage	
	Control	**Treated**	**Control**	**Treated**
Ranjit	0.87±0.03	0.52±0.01	0.76±0.02	0.68±0.01
Mahsuri	0.87±0.01	0.98±0.01	1.00±0.01	0.92±0.15
Kajoli chakua	0.85±0.01	0.76±0.01	1.20±0.01	0.97±0.02
Mean	0.86±0.01	0.75±0.23	0.98±0.22	0.85±0.15
t value		0.82		2.60
P value		0.49 NS		0.12 NS
SE (m)	0.01	0.13	0.12	0.08

The data represented are the mean of three replications ± Standard deviation

NS : Not significant

ANNEXURE V

Total soluble protein content (mg/g, fresh weight basis) of catalase extract prepared from rice leaves at three different growth stages

Variety/ Stage	Maximum tillering stage		Grain filling stage		Harvesting stage	
	Control	**Treated**	**Control**	**Treated**	**Control**	**Treated**
Ranjit	1.24±0.05	1.33±0.01	1.48±0.02	1.32±0.04	1.60±0.03	1.65±0.05
Mahsuri	1.48±0.01	1.55±0.02	1.51±0.03	1.39±0.03	1.63±0.03	1.67±0.06
Kajoli chakua	1.59±0.03	1.62±0.02	1.31±0.03	1.47±0.02	1.63±0.03	1.71±0.01
Mean	1.43±0.17	1.50±0.15	1.43±0.10	1.39±0.07	1.62±0.01	1.67±0.03
t value		-3.59		0.39		-4.71
P value		0.06 NS		0.72 NS		0.04*
SE (m)	0.10	0.08	0.06	0.04	0.01	0.02

The data represented are the mean of three replications ± Standard deviation

*Significant at 5% level of probability

NS : Not significant

ANNEXURE VI

Total soluble protein content (mg/g, fresh weight basis) of catalase extract prepared from brown rice at two different growth stages

Variety/ Stage	Grain filling stage		Harvesting stage	
	Control	Treated	Control	Treated
Ranjit	16.58± 0.62	15.16±0.28	24.08±0.38	25.00±0.50
Mahsuri	16.41±0.14	16.75±0.66	22.66±0.94	23.41±0.76
Kajoli chakua	16.50±0.66	16.16±0.38	23.58±1.46	23.75±1.39
Mean	16.49±0.08	16.02±0.80	23.44±0.72	24.05±0.83
t value		0.92		-2.70
P value		0.45 NS		0.11 NS
SE (m)	0.04	0.46	0.41	0.48

The data represented are the mean of three replications ± Standard deviation

NS : Not significant