5. DISCUSSION

5.1. Effluent Analysis

The physicochemical properties of the effluent (Table-1) showed that it is with alkaline pH, possessed high values of EC, BOD and COD and is rich in total dissolved solids. The effluent contains considerably high amounts of mineral nutrients. The Central Pollution Control Board (CPCB) and Indian Standard Institution (ISI) have formulated various standards for the disposal of industrial effluents. The Environment (Protection) Rules, 1986, clearly specify the effluent standards with respect to fermentation industry. These standards comprise the tolerance limit for the effluents discharged into inland surface water, land and marine coastal areas.

The approved tolerance limit of effluent irrigation on land for pH, suspended solids, total dissolved solids, chlorides and BOD are 5.5-9.0, 100 mg/L, 2100 mg/L, 600 mg/L, 500 mg/L respectively (ISI, 1974, CPCB, 2001). From the physicochemical analysis of the distillery effluent, it is clear that some treatment is essential to minimize the hazardous effect of the effluent before its discharge. Dilution of the effluent with water is a recommended procedure to meet the criterion of prescribed standards.

Presence of larger amount of essential nutrients like nitrogen, phosphorus, potassium etc. emphasizes the usage of this effluent as a substitute for the mineral fertilizer. The application of

Studies on the effect of distillery effluent on rice (Oryza sativa L.)
effluent on agricultural soil helps in the recycling of the nutrients and thereby helps to buildup the soil fertility. Besides these it also helps to minimize the present pollution problems. Similar conclusions were also made by Chauhan and Sharma (1997), Saxena, (2003), Sharma and Aggarwal, (2003), Chatterjee et al., (2003) and Sivaraman and Thamizhiniyan, (2005).

5.2. Germination Studies

Seed is the functional unit that contains an embryo which germinates and develops into the seedling and later into the plant. It is structurally complex with physiological specialization, closely associated with its development. Seed germination and growth are of vital importance for the continuation of plant life. In the physiological sense, germination begins with the imbibition of water and ends with the initiation of elongation by the embryonic axis, usually the radicle (Welbaum et al., 1998). Rice seed germination, according to Yung (1938), is the pushing of coleorhiza through the pericarp leaving a cavity in front of the root cap and by subsequent growth of the coleoptile under favourable conditions.

Hydration of the seed is basically the important requirement to initiate and trigger the intricate sequence of metabolism essential for germination and growth of the seedlings. The process involves a number of metabolic activities that are preceded by the absorption of water by the dry seed. This is a critical stage where the seeds and the seedlings are extremely sensitive to

Studies on the effect of distillery effluent on rice (Oryza sativa L.)
environmental stress. The presence of polluting agents in the environment of germinating seeds results in the deleterious effect on the germination and seedling growth (Kumar and Bhargava, 1998).

In the present study, concentrations up to 10% showed a significant increase in germination percentage and thereafter a reduction in both the varieties (Table-2; Figure-1). Another unique finding was the delay in the speed of germination in the higher concentrations in both the varieties. The result of the present study is in unison with the findings of Padhan and Sahu (1999), Misra and Pandey (2002), Tomer et al. (2002), Raina and Aggarwal (2003), Verma et al. (2004) and Mala and Babu (2005).

The process of germination starts with the imbibition of the medium supplemented for the germination. The seeds imbibe water to hydrolyze the reserve foods and to activate the enzyme system. The presence of high amount of various salts in the germinating medium may prevent or retard the absorption of water and cause toxicity to the embryo and endosperm. This osmotic absorption might be the limiting factor due to the high salt concentration of the effluent and cause delay in the germination (Bhatnagar et al., 1986; Vasanthy and Lakshamanaperumalasamy, 1998). The reduction in the germination percentage at higher concentrations may be due to the presence of excess amount of ions in the effluent that cause depletion of acids from tricarboxylic

Studies on the effect of distillery effluent on rice (Oryza sativa L.)
acid cycle which reduces the respiration rate (Kirkbly, 1968). Ching, (1972) opined that the germinating seeds are with high respiration rate and require high amount of dissolved oxygen for the liberation of energy and due to high salt content, the oxygen availability is reduced resulting in the low germination percentage. This view was also supported by Ajmal and Khan (1984b), Rani et al. (1990) and Sundaramoorthy et al. (2001). Mala and Babu (2005) reported that the increased amount of the total solids in the effluent enhanced the salinity and conductivity of the solutes which in turn disturbed the osmotic relationship between the seed and water, thus restricting the energy supply through aerobic respiration.

The optimum level of nutrients in the diluted effluent might have provided favorable conditions for germination by increased imbibition and osmosis resulting in the hydration of sub-cellular organelles. This resulted in the increased metabolic and enzyme activities which in turn enhanced the germination percentage. Similar conclusions were also made by Baruah and Das (1998), Ready and Borse (2001) and Verma et al. (2004).

The importance of the embryonic axis on reserve mobilization during germination emphasized the breakdown of the carbohydrate reserves catalyzed by the enzymes (Bewley and Black, 1983, 1985; Mayer and Poljakoff Mayber, 1989). In the present study, concentrations upto 5% showed an increase and
thereafter a reduction was observed in the mobilization efficiency percentage in both the varieties (Table-2, Figure-1). The result is in agreement with the findings of Pretorius et al. (1998) and Sundaramoorthy et al. (2001).

Increased activity of the hydrolyzing enzymes such as protease, amylase etc. mobilized the major food reserves and provided the energy for the continued growth of the embryonic axis resulting in the increase in the mobilization efficiency percentage in the lower concentrations. In the higher concentrations, the osmotic relationship between the seed and water was inhibited due to excess amount of salts resulting in the reduced water uptake. Besides, the germinating seeds get low amount of oxygen in the dissolved form and this reduces the energy supply to carry out the effective mobilization (Singh et al. 1985, Rani et al. 1990).

Seedling growth was increased in the lower concentrations but in the higher concentrations it was decreased. Root length showed an increase in growth upto 10% and in 30% there was no root emergence in MO 16 while in MO 19, the increase was in concentrations upto 5% and there was no root emergence from 20% onwards (Table-3; Figure-2). A unique finding in this experiment was that, there was shoot emergence but no root emergence in the higher concentrations, which was quite contradictory to the findings of Yung (1938), but the same findings

Studies on the effect of distillery effluent on rice (Oryza sativa L.)
as in this experiment was made by Behera and Mishra (1982).

Shoot length showed an increase in concentrations upto 10% in MO 16 while in MO 19 this was upto 5% and thereafter a gradual reduction with the increase in concentrations in both the varieties (Table-3; Figure-2). The result of the present study is in agreement with the findings of Baruah and Das (1998), Padhan and Sahu (1999) and Pandey and Pandey, (2002).

The enhancement in shoot and root growth in the lower concentrations may be due to the presence of optimum concentrations of the essential nutrients that are needed for the plant growth. The increased mobilization of the hydrolyzed products of the storage reserves is used by the embryonic axis for anabolic processes and growth (Srivastava, 2005).

The reduced growth in the higher concentrations might be due to the lower amount of the dissolved oxygen because of the presence of the high salt concentrations in the effluent which reduced the energy supply through anaerobic respiration (Behera and Mishra, 1982, Saxena et al. 1986). The excess accumulation of chloride ions in the higher concentrations of effluent might have reduced the turgor pressure inside the cells resulting in the reduction in seedling growth (Sheoran and Garg, 1983).

The fresh weight and the percentage dry matter production of the seedlings increased with the advancement of germination indicate the physical process of growth. In the present study, the
fresh weight and the percentage dry matter production increased in the lower concentrations while from 20% onwards, there was a decrease in both the varieties (Table-4; Figure-3). Similar results were also reported by Vijayakumari and Kumudha (1990), Om et al. (1994), Vasanthy and Lakshamanaperumalasamy (1998), Sundaramoorthy et al. (2001) and Rajeswari et al. (2005).

The increase in fresh weight in the lower concentrations may be due to the rapid hydrolysis and translocation of reserve food materials throughout the young shoots and roots, which in turn might have made the cell’s water potential more negative. This might have helped the seedling for more water uptake for cell expansion and maintenance of higher turgor pressure (Salisbury and Ross, 1995). The increase in dry weight can be attributed to the rapid hydrolysis of reserve food materials by gluconeogenic enzymes and because of higher soluble sugar content and soluble proteins in the endosperm (Pandey and Sinha, 1995).

The retardation in the seedling growth may be because of the higher concentrations of salts and ions in the effluent which might have reduced the uptake of water. The enhanced salinity and conductivity of the effluent might also have reduced the supply of energy through aerobic respiration restricting the growth (Rani et al., 1990). The increased concentrations of the effluent might have affected the protoplasmic balance of the cells that in turn might have reduced the water uptake resulting in the reduction in

Studies on the effect of distillery effluent on rice (Oryza sativa L.)
the growth of the seedlings (Vijayakumari et al., 1993).

Shoot root ratio, both on length basis and dry weight basis, showed a gradual increase along with the increase in the effluent concentrations. No values were obtained at 30% in MO 16 and 20 and 30% in MO 19 because of the absence of root emergence (Table-5; Figure-4). The result of the present study is in unison with the findings of Behera and Mishra (1982), Sahai et al. (1983), Om et al. (1994) and Pandit et al. (1996).

The increase in the shoot root ratio might be due to the imbalance in the root and shoot growth under the effluent stress. The imbalance may be due to the absorption of monovalent cations by the roots and its accumulation in the vacuoles of the root cells preventing the translocation of the salts leading to the retardation of growth (Solov’ev, 1967). This agrees with the present findings. Behera and Misra (1982) suggested that the adverse effect on the rice seedlings are due to many basic components present in the effluent. They also opined that the retardation of root growth might have been due to the presence of polycations in the effluent.

Seedling vigour index on length basis and dry weight basis showed an increase in the concentrations upto 10% and thereafter a reduced vigour index was observed (Table-6; Figure-5). This result is in agreement with the studies of Rajannan and Oblisami (1979), Jabeen and Susan Abraham (1998) and Mariappan and Rajan (2002).
The increase in seedling vigour index in lower concentrations can be attributed to the reduction in the physiological deterioration of seeds maintaining membrane integrity of bio-organelles, in terms of counteracting free radical formation and lipid peroxidation reactions (Basu et al., 1985). Tao-Hanzhi et al. (1995) attributed the increased vigour index to the increased water uptake, respiratory rate, water retention capacity, catalase activity, high total soluble sugar, and soluble protein in the cotyledon.

In the higher concentrations, the membrane of the embryonic axis has increased the permeability that permits the entry of salts in higher degrees and causes a reduction in the vigour of the seedlings (Khedkar and Dixit, 2005).

5.3. Growth and Development - Field Studies

In the present study, it was observed that the treatments had a significant effect on the vegetative as well as reproductive growth in both the varieties. The treatments upto 30% showed an increase in most of the parameters studied with a varietal difference.

5.3.1. Growth attributes

Growth of the organism is the increase in volume, weight, cell number, amount of protoplasm and complexity of the cell and organism (Salisbury and Ross, 1995). Vaadia et al. (1961) reported that the increase in growth is expressed by the enlargement of cells and it is brought about by the action of water.
In the present study, the root length and shoot length increased with the growth period. The observations showed that the concentrations up to 40% showed an increase in root and shoot length while a reduction in growth was observed in 50% in both the varieties (Tables-14 & 15; Figures 13-16). These results are in agreement with the findings of Kannabiran and Harilal (1998), Misra and Pandey (2002), Raina and Aggarwal (2003), Rajeswari et al. (2005) and Singh et al. (2006).

The increase in plant growth at lower concentrations might be due to the beneficial effects of salts present in the effluent that play a vital role in the growth and development of plants. The adequate supply of the inorganic ions present in the effluent might have increased the rate of photosynthesis leading to the increased growth rate. The uptake and biosynthesis of solutes by lowering the water potential might have resulted in the increased growth of root and shoot cells (Salisbury and Ross, 1995).

The growth retardation in the higher concentrations may be due to the excess salt contents in the effluent. Bernstein and Hayward (1958) pointed that the increased accumulation of soluble salts and its increased osmotic pressure is related to the high degree of growth inhibition. Eaton (1942) and Hayward and Wadleigh (1949) suggested that the reduction in the water availability induced high osmotic pressure of the root medium leading to the retardation of growth in plants.

Studies on the effect of distillery effluent on rice (Oryza sativa L.)
Fresh weight also increased with the growth period. Concentrations upto 30% showed an increase and thereafter a reduction was observed in all the phases of growth (Table-17; Figures-18 & 19). The percentage dry matter production showed a linear relationship with growth. Here also concentrations upto 30% showed an increase in all the phases of growth in both the varieties (Table-18; Figures-20 & 21). These results are in agreement with Sahai and Srivastava (1986), Shinde et al. (1988), Kumar and Bhargava (1998), Ramana et al. (2002) and Elayarajan and Jothimani (2004).

Behera and Misra (1982) observed that the fresh weight and dry weight was in inverse relationship with the effluent concentration. In the present study also a similar relationship was observed in both the varieties. The optimum level of nutrients present in the diluted effluent stimulated the growth by increased uptake of nutrients, translocation, synthesis of cellular constituents and hydrolysis of macromolecules. In the diluted effluent, the ample supply of inorganic nutrients influenced the metabolic processes resulting in the increased fresh weight and dry matter production.

The presence of excess salts in the higher concentrations of effluent affected the plant growth by developing more negative water potential and specific ion toxicity resulting in the reduction of plant growth. Om et al. (1994) opined that the reduction in the

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Studies on the effect of distillery effluent on rice (*Oryza sativa* L.)
fresh and dry weight in the higher concentrations of the effluent is due to the increase in BOD and COD, which might have damaged the cells due to the reduced dissolved oxygen.

Leaf area and chlorophyll content are the two major factors that influence the photosynthetic efficiency of a plant (Ashraf and Ali, 1998, Taiz and Zeiger, 2003). The correlation between the leaf area and yield is also suggested by Alluwar and Deotale, (1991). In this study, leaf area showed a promotive effect in the concentrations up to 30% and thereafter a reduction was noticed in both the varieties (Table-19; Figures- 22 & 23). The result is in agreement with Srivastava and Sahai (1987) and Singh and Misra (1987).

The promotive effect in the lower concentrations may be due to the increased cell division and cell expansion, which in turn has contributed to the high rate of photosynthesis and relative growth rate. In the higher concentrations, the excessive amount of soluble salts might have created a reduction in turgor pressure resulting in a water stress which in turn causes a reduction in leaf area. Srivastava and Sahai, (1987) stated that the toxicity of the effluent is proportional to the amount of salts which has got an impact on leaf area.

Shoot root ratio is directly proportional to the growth and dry matter assimilation (Tables- 20 & 21; Figures – 24 - 27). In the present study, shoot root ratio on length basis showed a varying effect
in MO 16 whereas in MO 19, higher concentrations showed a reduction. Shoot root ratio on dry weight basis showed an increase in concentrations upto 30% and thereafter a reduction in both the varieties. The result is in unison with the findings of Sahai et al. (1983) and Pandit et al. (1996).

The increase in shoot root ratio can be attributed to the high shoot growth rather than root growth. The promotive effect of shoot growth over root growth in the present study may be due to the increased plasticity of cell wall with the increased production of reducing sugars which in turn caused increased water uptake and growth. Similar conclusion was also made by Thomas et al. (1981).

The reduction in the shoot root ratio in the higher concentrations might be due to the reduction in above and below ground biomass caused by the higher levels of chlorides and sulphates which interfere in the uptake of essential nutrients. The elevated concentrations of ions in the effluent may inhibit the activity of IAA oxidases leading to the increase in IAA concentration resulting in reduced root growth (Behera and Misra, 1982).

Relative growth rate is used for correlating the growth over a period of time. It is also related with the nitrogen content of the plants (Ingestad and Agren, 1992). Net primary production is a measure determined by the dry matter production of plant. In the present study, relative growth rate showed an increase in

Studies on the effect of distillery effluent on rice (Oryza sativa L.)
concentrations up to 30% in both the varieties (Table-22; Figures -28 & 29). Similar results were also observed in net primary production also (Tables-29, 30; Graphs-20, 21). The results are in agreement with that of Srivastava and Sahai, (1987), Ramana et al. (2002), Banerjee et al. (2004).

The increase in relative growth rate and net primary production in the lower concentrations is because of the overall promotive effect on length and weight of the plant. The higher rate of photosynthesis in the plants treated with lower concentrations of effluent might have resulted in the increased dry matter production and this indicated the increased relative growth rate and net primary production. This may be due to the active uptake of nutrients and minerals from the effluent which in turn might have led to the increased metabolism.

The decrease in relative growth rate and net primary production in the higher concentrations can be attributed to the accumulation of salts in the rooting zone that lowers the water potential of the soil and also creates specific ion toxicity. Victoria (1996) explained that the reduction in relative growth rate and net primary production in the higher concentrations may be due to the deficiency in oxygen in the effluent and this might have reduced the absorption of water and nutrients.

The net assimilation rate is a measure of the amount of photosynthetic product going into the plant and it is an estimate of

Studies on the effect of distillery effluent on rice (*Oryza sativa* L.)
net photosynthesis (Noggle and Fritz, 2002). In the present study, net assimilation rate showed an increase in concentrations upto 30% in both the varieties (Table- 24; Figures – 32 & 33). Similar results were reported by Sahai et al. (1985) and Mary and Vivekanandan (1990).

The increased net assimilation rate in the lower concentrations may be due to the increase in net photosynthesis and the faster utilization of assimilates resulting in the increase in biomass. This agrees with the studies of Thorne and Evans (1964) and Sweet and Wearing (1966). In the higher concentrations, the increased salt content of the effluent might have created an inadequate utilization of the photosynthates leading to the reduced growth. The influence of increased specific ion concentrations in the effluent might have disrupted the membrane integrity and other physiological and biochemical processes leading to the growth retardation (Inananga et al., 1979).

Leaf area ratio is a measure of the proportion of the plant that is engaged in photosynthetic process and is reported as the area of leaf surface in square centimeter per gram dry weight (Noggle and Fritz, 2002). Leaf area ratio showed a gradual reduction as the growth progressed. This decrease suggests the increased mobilization of photosynthates from leaves as the growth proceeds (Friend, 1966). In the present study, 40% and 50%

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Studies on the effect of distillery effluent on rice (*Oryza sativa* L.)
showed a comparative increase in both the varieties (Table -25; Figures -34 & 35).

The increase in leaf area ratio in the higher concentrations might be due to the reduction in the rate of photosynthesis and relative growth rate (RGR) which in turn has contributed to the decreased dry matter production. A similar conclusion was made by Murthy et al. (1986). The reduction in the leaf area in 40 and 50% might also have contributed to the increase in the leaf area ratio. Friend (1966) suggested that, along the progress of growth, the decrease in leaf area ratio may be due to the increased mobilization of photosynthates from the leaves.

5.3.2. Yield attributes

The effect of distillery effluent on yield attributes such as productive tillers, panicle length, panicle weight and 1000 seed weight were studied (Tables- 26 & 27; Figures – 36 & 37). In the present study, an increase in the yield attributes were noticed in plants irrigated with the concentrations up to 30% while the higher concentrations showed a decrease in both the varieties. The results are in agreement with the findings of Kumar and Bhargava (1998), Rampal and Dorjey (2001), Raina and Aggarwal (2003), Chatterjee et al. (2003) and Medhi et al. (2005).

The promotion of yield attributes in the present study is due to the presence of the growth promoting nutrients like nitrogen, phosphorus, potassium in the effluents which has promoted the
plant growth and its yield. The increased uptake of nutrients in the
diluted effluent made a better source and sink relationship which
contributed to the increased dry matter production resulting in the
increase in yield. The increased yield characters may also be
attributed to the improved seed filling through increased
translocation and accumulation of photosynthates from source to
sink at the grain filling stage. This can be further substantiated by
the higher values of net assimilation rate and dry matter
production.

Zalawadia and Raman (1994) stated that the significant
increase in yield may be due to the considerable quantities of
major, secondary and micronutrients in the effluent. The increase
in the seed weight may be due to the synthesis and deposition of
storage molecules from the small precursor molecules of the
parent plant i.e., starch, proteins, fats, phytin etc. in the
endosperm (Noggle and Fritz, 2002).

The grain yield of the present study is in unison with the
results of Chatterjee et al. (2003) and according to them, this may
be due to the presence of higher amount of phosphorus in the
distillery effluent. The variation between the varieties may be due
to the difference in uptake of potassium. Medhi et al. (2005)
opined that the increase in panicle length and seed weight is by the
improvement of chemical properties of the soil because of the
presence of the optimum level of the essential nutrients in the

Studies on the effect of distillery effluent on rice (Oryza sativa L.)
effluent. Similar conclusions were also made by Kumar and Bhargava (1998) regarding the increase in spikelet weight and grain weight in wheat treated with sugar mill effluent. Jeyabhaskaran and Sreeramulu (1998) explained that the increase in the yield parameters is due to the increased vigour of rice plants in the seedling phase of growth.

The decrease in yield at higher levels of effluent might be due to the higher osmotic concentration, increased accumulation of salts and high BOD load in the effluent. The reduction in the net assimilation rate and leaf area might have caused a reduction in the rate of photosynthesis and this in turn reduced the yield attributes. Grattan and Grieve (1994) correlated the reduction in yield components with the nutritional imbalance due to excess salt concentrations. Medhi et al. (2005) ascribed the reduction in yield to the high amount of inorganic substances and also due to the toxic effect created by the higher concentrations of nutrients in the effluent.

5.4. Metabolic Drift during different stages of growth

5.4.1. Carbohydrate metabolism

Carbohydrates are the principal respiratory substrates and are the important components of storage and structural materials in plants. They exist as free sugars and polysaccharides. The basic units of carbohydrates are monosaccharides, which join in long linear or branched chain to form polysaccharides. These also act as
the metabolic reserves in plants (Lehninger, 1996; Jain, 2004). The carbohydrates are usually translocated as sucrose and to some extent as starch. The shielding role of sugars against stress was reported by many workers (Singh et al. 2002; Ameta Suresh et al., 2003; Rajeswari et al., 2005).

In the present study, seedlings after 96 hours of germination, showed an increase in the total and reducing sugar contents in concentrations upto 10% and thereafter a reduction was observed in both the varieties (Table-7; Figure-6). In the vegetative and reproductive phases of growth, concentrations upto 30% showed an increase and thereafter a reduction in both the varieties and more sugar content was in the reproductive phase than in the vegetative phase (Tables- 28 & 29; Figures– 38 & 39). The results are in agreement with that of Wadkar et al. (1984), Singh et al. (2002), Ameta Suresh et al. (2003), Rajeswari et al. (2005), Sivaraman and Thamizhiniyan (2005) and Singh et al. (2006).

During seed germination, the hydrolysis of starch takes place and soluble sugars thus formed are metabolized and transported in the seedlings and used as source of carbon and energy. The increased sugar content in the lower concentrations give a reflection of higher potential to accumulate sugars at a faster rate and provide a protective function through osmoregulation, hence this accumulation must not be taken as its non utilization but may be due to the better balance between the

Studies on the effect of distillery effluent on rice (Oryza sativa L.)
anabolic and catabolic processes (Singh, 1980). A disturbed starch metabolism and poor translocation of sugars might be the reason for the reduction of sugars in the concentrations above 10%. Similar conclusion was also made by Sivaraman and Thamizhiniyan (2005).

During the vegetative phase, the increased assimilating area led to the increased synthesis of photosynthates (Leopold and Kriedermann, 1985). These photosynthates are translocated to the growing regions of the plant resulting in the increased dry matter production. The sugars produced during photosynthesis also get converted to starch and are accumulated in various sink tissues like stems. After panicle initiation, accumulation of available carbohydrates such as starch and sugars increases in the leaf sheath and culm base because of the reduction in growth of the vegetative organs (Murata and Matsushima, 1975). This attributes to the increased total and reducing sugar content in the reproductive phase.

As the growth advanced, due to photosynthesis, the production of sugars and their conversion into other carbohydrates occur. However, since the sugars are utilized only for osmotic adjustments, inter-conversions into other cellular polysaccharides could not occur; thus there was a lesser availability of those cellular constituents probably for cellulose, which results into retardation of extension or elongation of root and shoot as

Studies on the effect of distillery effluent on rice (Oryza sativa L.)
observed by Kaufmann (1968). It was suggested that sugars are compatible cytosolutes, since many of them have effects on cytoplasmic enzymes and could be incompatible in high concentrations of salts (Hawker and Walker 1978). The increased quantity of salts in the higher concentrations in turn enhanced the salinity and conductivity of the solutes resulting in the decrease in the sugar content. The influence of increased specific ion concentrations in the effluent might also have disrupted the membrane integrity and other physiological and biochemical processes (Inananga et al. 1979). The presence of high amount of total solids in the effluent might have disturbed the osmotic relations and created a water stress and this water stress might have decreased both photosynthesis and consumption of assimilates (Taiz and Zeiger, 2003).

Starch is the major food reserve in rice grains and is used as the energy source. During germination, the mobilization of starch commences soon after the emergence of the radicle, which is preceded by the imbibition of water (Mukherji and Ghosh, 2006). Starch is the most important and abundant storage polysaccharide accumulating in plants. It accumulates in chloroplast where it is formed directly from photosynthesis and allows storage of complex sugars (Salisbury and Ross, 1995; Srivastava, 2005).

In the present study, the seedlings showed an increase in starch content along with the increase in effluent concentration in

Studies on the effect of distillery effluent on rice (Oryza sativa L.)
both the varieties (Table- 8; Figure- 7). In the vegetative and reproductive phases of growth, increased starch content was found in concentrations upto 30% and thereafter a reduction (Table- 30; Figure- 40). Starch content was more in the vegetative phase over the reproductive phase. The result of the present study is in agreement with the observations of Kannabiran and Harilal (1998), Lakshmi and Sundaramoorthy (2000), Singh et al. (2002) and Sivaraman and Thamizhiniyan (2005).

During germination, the embryo secretes gibberellins which in turn induce the secretion of the hydrolytic enzymes such as amylase that break down the starch to simple sugars (Reddy and Reddi, 2003). The increase in starch content in the higher concentrations may be due to the reduction in the amylase activity. This can be attributed to the partial inhibition of internal hormone activity which is responsible for the synthesis and activity of α-amylase. Moreover, the water stress developed from the negative water potential might have restricted the imbibition of water in the higher concentrations of effluent. Kaur et al. (2005) opined that, the conversion of starch to sucrose is inhibited during water deficit and salt stress leading to the decreased transport of sucrose to the growing tissues. Here also, the effluent induced a similar stress resulting in the reduced utilization of starch.

During the vegetative phase, the rate of photosynthesis per unit area increased due to the increase in leaf area and number of
tillers and this in turn increased the assimilation and synthesis of starch. In most circumstances, the rate of photosynthesis is greater than the rate of translocation and the net accumulation of carbohydrate in the form of starch occur in the leaves. This also resulted in the increase in starch content in the vegetative phase (Reddy and Reddi, 2003). During the reproductive phase, the photosynthates are translocated from the source to the sink. This resulted in the reduction in the starch content in the leaves in the reproductive phase. The increased starch content in the lower concentrations might be due to the promotive effect of the mineral salts present in the effluent. Similar conclusion was also made by Rajeswari et al. (2005). Victoria (1996) suggested that the increase might be due to the absorption and transportation of nitrate present in the effluent.

The reduction in starch content in the higher concentrations may be due to the toxic effect of salts accumulated in the effluent. This might have delayed the starch synthesis and induced a negative feedback on carbohydrate mobilization from the stems and leaf sheaths to the grains (Wardlaw, 1990). Wilson (1998) linked the reduction in starch content with the decreased photosynthetic rate in the effluent treated plants. Kannabiran and Harilal (1998) opined that the decrease in starch content may due to the lowered activity of phosphorylase and increased activity of β-amylase and invertase. The reduced starch content in the plants

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Studies on the effect of distillery effluent on rice (Oryza sativa L.)
treated with higher concentrations of effluent implied the deranged starch metabolism and poor translocation of sugars and other metabolites to the growing parts (Lakshmi and Sundaramoorthy, 2000).

Amylases are the chief hydrolytic enzymes associated with the carbohydrate metabolism that break down the starch. The hydrolysis of starch to dextrins and maltose are regulated by the activity of amylases (Mukherji and Ghosh, 2006). The imbibition of water stimulated the de novo synthesis of amylases in the germinating seeds resulting in the break down of starch to glucose (Noggle and Fritz, 2002).

Amylase activity in the seedlings showed a gradual reduction along with the increase in concentrations in both the varieties (Table- 11; Figure- 10). An increase in the amylase activity was observed in concentrations upto 20% and 30% in the vegetative and reproductive phases respectively (Table- 35; Figure- 45). Increased amylase activity was observed in the reproductive phase over the vegetative phase. Similar results were observed by Singh et al. (1994) and Kamleshnath et al. (2004).

During germination, the increased activity in the lower concentrations might be due to the stimulatory effects of the salts present in the diluted effluent, which has reduced the water potential and helped in the increased hydration of the cell organelles. This increase in turn stimulated the synthesis of

Studies on the effect of distillery effluent on rice (Oryza sativa L.)
amylases that participated in the starch degradation (Leopold and Kriedemann, 1985). The reduced activity could be due to the water deficit created by the increased salt content of the effluent. Higher level of starch accumulation due to the reduction in amylase activity was observed by Lin and Kao (2000). Kaur et al. (2005) reported that the excess salts exerted a significant reduction on de novo synthesis of enzymes of carbohydrate metabolism leading to the reduced synthesis of sucrose and thereby decreased the seedling growth.

In the vegetative phase, amylase activity increased along with the increase in starch content which can be attributed to the enhanced rate of photosynthesis. Here, the higher levels of amylase activity may be used for the rapid hydrolysis of starch which in turn may be used for the rapid vegetative growth. In the reproductive phase, the growth activities are generally retarded and hence the productivity, hydrolysis and utilization of the metabolites are reduced. The depression in the enzyme activity in the higher concentrations might be due to the presence of excess salts in the effluent this in turn reduce the hydrolysis of the substrate. Similar conclusion was made by Kamleshnath et al. (2004). The increase in the lower concentrations emphasizes the promotive effect of the effluent Greenway and Munns (1980) reported that compartmentalization of electrolytes prevents the

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Studies on the effect of distillery effluent on rice (*Oryza sativa* L.)
enzymes from direct injury of salts but enzymes as such may not tolerate salt concentrations.

Invertase is one of the enzymes that are directly related with the carbohydrate metabolism. The major reaction of sucrose degradation is by irreversible hydrolysis to free glucose and fructose by the enzyme invertase (Salisbury and Ross, 1995). Invertase also contributes in the initial step of sucrose metabolism in the developing rice grain. In addition, the presence of invertase also suggests the possibility of sucrose hydrolysis during its movement into the endosperm (Tarplay et al., 1994). Invertase has a bioregulatory control in the mobilization of carbohydrates from the source to the generative organ formation (Asthir and Singh, 1995) and during responses to environmental stresses (Taiz and Zeiger, 2003).

In the seedling stage, concentrations upto 5% showed an increase in the specific activity and thereafter a reduction was observed in both the varieties (Table- 11; Figure- 10). In the vegetative and reproductive phases of growth, concentrations upto 20% showed an increase (Table- 36, Figure- 46). Increased invertase activity was noticed in the vegetative phase over the reproductive phase. The observations are in corroboration with that of Hawker and Walker (1978), Gopalakrishnan (1980), Singh et al. (1994) and Kaur et al. (2005).

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Studies on the effect of distillery effluent on rice (Oryza sativa L.)
The utilization of sucrose can be related with the activity of sucrose splitting enzymes such as invertase. In addition, genes for invertase and sucrose synthase are often expressed at different times during sink development (Taiz and Zeiger, 2003). After panicle initiation, a reduction in the growth of vegetative parts was observed with the accumulation of sugars. This may be due to the low activity of invertase in the reproductive phase. Similar conclusion was also made by Bhatia and Singh, (2001). According to Tarplay et al. (1994), the reduction in invertase activity is a prerequisite for sucrose accumulation in any plant tissue.

In the higher concentrations of effluent, the excess amount of salts created an adverse environment and the plants might have utilized their carbohydrate resources to synthesize sugars, which are osmoregulatory in function. The decreased invertase activity in the higher concentrations may cause the reduced conversion of total sugars to hexoses which in turn reduced the formation of sugar nucleotides for cell synthesis and growth (Kaur et al., 2005). Hawker and Walker (1978) also noticed a decrease in invertase activity along with the decrease in the sugar content in the higher salt concentrations. This can be attributed to the general decrease in the total metabolic activities caused by increased salt concentrations in the effluent.
5.4.2. Protein metabolism

Protein metabolism, one of the fundamental events of the cell, is in a continuous state of flux between the synthesis and breakdown. Proteins play a vital role in the growth and metabolism of the plant. Aminoacids, the precursors of the protein synthesis, are the initial products of nitrogen assimilation. The aminoacids formed by the metabolic activities in the cytoplasm interact with t-RNA and forms a complex. It is then oriented to the ribosome and translated to the proteins. Large amount of proteins may remain stored in plant tissues as a source of energy and nitrogen to be utilized during the period of growth (Mukherji and Ghosh, 2006). Proteins, one of the major food reserves in plants, during germination, mobilized and metabolized to produce aminoacids by the activity of the enzyme, protease (Stiles, 1999; Noggle and Fritz, 2002). Bewley and Black (1978) stated that protein synthesis is a pre-requisite for the radicle emergence.

In the present study, the seedlings after 96 hours of sowing showed an increase in protein content along with the increase in effluent concentrations with the maximum in 30% in both the varieties (Table- 9; Figure- 8). In the vegetative phase, concentrations upto 30% showed an increase in MO 16, while in MO 19, it was upto 20%. In the reproductive phase, concentrations upto 20% showed an increase in both the varieties. Increased protein content was observed in the reproductive phase over the

Studies on the effect of distillery effluent on rice (Oryza sativa L.)
vegetative phase (Table-31; Figure-41). The results are corroborative with the findings of Ameta Suresh et al., (2003), Rajeswari et al., (2005), Singh et al., (2005) and Sivaraman and Thamizhiniyan (2005).

During germination, the storage proteins are hydrolyzed by the enzyme protease to form aminoacids that provide early growth and development (Noggle and Fritz, 2002, Mukherji and Ghosh, 2006). In the lower concentrations, reduced protein content was observed in the seedlings. This may be due to the increased protease activity in the seedlings grown in the lower concentrations. Karande (1990) also observed a similar reduction in the protein content and he suggested that this decrease was evidenced by the increased activity of protease leading to the synthesis of aminoacids. The increased protein content in the higher concentrations can be attributed to the reduced utilization of the stored proteins. Mala and Babu, (2005) suggested that in the higher concentrations, there is a reduction in the oxygen supply due to the increased total solids in the effluent and this might have reduced the respiration rate and reduced the protein breakdown. Similarly the water stress developed in the higher concentrations, reduced the uptake of water and thereby lowered the enzyme activity. This also might be a reason for the reduced breakdown of the proteins.

Studies on the effect of distillery effluent on rice (*Oryza sativa* L.)
In the present study, protein content showed a reduction in the vegetative phase over the reproductive phase. In the vegetative phase, the effective utilization of the synthesized proteins take place and this can be evidenced by the active growth and increased accumulation of dry matter. The protein manufactured in the leaf is utilized in some other tissues and so it is translocated from the seat of synthesis (Stiles, 1999). This may also be a reason for the reduced protein in the leaves during the vegetative phase.

During the reproductive growth, the reduction in the utilization enhanced the accumulation of the synthesized proteins. In the cereal grains, as starch is the principal storage reserve, the mobilization of synthesized protein from the source to the sink is reduced. This also results in the accumulation of reserve protein (Leopold and Kriedermann, 1985, Noggle and Fritz, 2002). The accumulation of synthesized protein may be due to the protective effect of stress hormone proline, which gets accumulated in the higher salt concentrations (Rantein et al. 2002).

In the vegetative and reproductive phases of growth, the increased protein content in the lower concentrations can be attributed to the absorption of minerals especially nitrogen, phosphorus and potassium present in the effluent. This is evidenced by the increased tissue nutrient content in the lower concentrations. Stiles (1999) reported that potassium is essential
for the synthesis of proteins leading to the increased cell division. It also helps in the translocation of the food materials. Victoria (1996) ascribed the increase in protein content in the lower concentrations may be due to the absorption and transportation of the nitrate in the effluent. In the present study also the increased uptake of nitrogen and potassium might have increased the protein synthesis in the lower concentrations.

In the higher concentrations, the decrease in protein content can be directly correlated with the growth of the plant as measured by the length and weight. In the higher concentrations the reduced protein content may be due to the presence of increased strength of various cations and anions in the effluent. Uprety and Sarin (1976) opined that the disturbance in aminoacid metabolism may be the reason of the reduction in protein synthesis. Helel and Mengel (1979) observed that the mutilation of enzyme involved in protein metabolism is an important aspect of salt stress which is possibly induced by the disturbance of sodium-potassium balance. Similar conclusion can be made in the present study also due to the higher levels of salt in the effluent.

Seedlings, after 96 hours of sowing, showed a reverse trend in the free aminoacid content as that of the protein, with the maximum reduction in 30% in both the varieties (Table- 9; Figure-8). In the vegetative and reproductive phases, concentrations upto 30% showed an increase in both the varieties. Increased free

Studies on the effect of distillery effluent on rice (*Oryza sativa* L.)
aminoacid content was observed in the reproductive phase over the vegetative phase (Table- 32; Figure- 42). The results are in unison with the findings of Taghavi and Vora (1994), Khan and Jain (1995), Pandey and Neraliya (2002), Saxena (2003), Patil and Chaudhari (2005) and Sivaraman and Thamizhiniyan (2005).

Protein hydrolysis is always associated with the increase in free aminoacids (Irigoyen et al., 1992). Here also, the seedlings showed an inverse relationship between the aminoacid and protein content. The rise in aminoacid content in the lower concentrations is due to the increased activity of protease which suggests that the proteins are in a continuous state of turnover and the aminoacids newly incorporated into proteins are not in association with those resulting from protein breakdown (Bidwell, 1979). In the higher concentrations, the reduction in aminoacid content might be due to the reduced proteolysis. This may be due to the water stress created by the effluent. Similar conclusion was made by Patil and Choudhari (2005).

The low aminoacid content observed at the vegetative phase can be attributed to the rapid utilization of the soluble aminoacid for the active growth. The increased activity of protease can be considered as an evidence for this finding. The increased aminoacid content in the reproductive phase might be due to the reduced protease activity and low rate of utilization of the synthesized

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Studies on the effect of distillery effluent on rice (Oryza sativa L.)
proteins. Lahiri et al. (1987) suggested that the accumulation of free amino acids can be related to the decrease in protein content.

Similar to the protein content, in the lower concentrations, increased free amino acid content was observed in the vegetative as well as the reproductive phases of growth. This increase can be attributed to the promotive effect of the dissolved solids present in the effluent. Pandey and Neraliya (2002) suggested that this increase might be due to the stimulation in protein synthesis by increased nitrogen metabolism. It is evident that amino acid synthesis is directly proportional to the carbohydrate availability (Mayer and Anderson, 1959) and in the lower concentrations increased production of carbohydrates might have contributed to the increased synthesis of amino acids.

Higher concentrations of the effluent made a reduction in amino acid content. This might be due to the presence of toxic cations and anions in the effluent. The reduced synthesis of carbohydrates and inadequate supply of $O_2$ in the higher concentrations might have reduced the synthesis of amino acids. Accumulation of ammonium ions in tissues under stress also result in the reduced synthesis of amino acids and proteins (Mayer and Anderson, 1959). Moreover the water stress created by the effluent might have also reduced the free amino acid content. Pennar and Ashton (1967) also noticed a similar reduction in amino acid content and they opined that it can be attributed to the inhibitory

Studies on the effect of distillery effluent on rice (Oryza sativa L.)
effect of protease activity. Levitt (1972) correlated the decrease in aminoacid content with the denaturation of enzymes involved in aminoacid and protein synthesis. According to Wilson (1998), concentrated industrial effluents may induce severe stress and this eventually damages the subcellular organelles disrupting their specialized functions such as protein and aminoacid synthesis.

Protease are a group of enzymes that catalyses the hydrolysis of protein molecules into smaller peptide fractions and amino acids. Seedlings showed an increased protease activity in concentrations upto 10% in both the varieties (Table-12; Figure-11). Concentrations upto 20 and 30% showed an increase in the protease activity in the vegetative and reproductive phases of growth respectively. Protease activity was more in the vegetative phase over the reproductive phase (Table- 37; Figure- 47). Similar results were observed by Karande (1990), Singh et al. (1994), Lata (2000).

Proteases are hydrolytic enzymes that synthesized during seed maturation and by the hydration it gets activated resulting in the breakdown of proteins (Taiz and Zeiger, 2003). Ihle and Dure (1972) suggested that the high activity of some enzymes like protease during germination is due to the presence of preformed m-RNA in the seeds. In the lower concentrations, the increased activity of the enzyme protease mobilized the protein reserve and provided the continued growth of the embryonic axis. The

Studies on the effect of distillery effluent on rice (*Oryza sativa* L.)
increased activity of protease was evidenced by Karande (1990) which led to the synthesis of amino acids by utilizing the reserve proteins. This can be correlated with the present findings. In the higher concentrations, the water stress arising from the elevated osmotic potential might have reduced the uptake of water during germination. Moreover, the salts present in the effluent might have induced disturbances in the normal metabolism of protease (Sheoran and Garg, 1979).

In the vegetative phase, the increase in protease activity can be correlated with the active utilization of the synthesized proteins leading to the increased growth and dry matter accumulation. Stiles, (1999) reported that, by the action of protease enzyme, the large immobile protein molecules are converted to amino acids which are more diffusible substances to provide translocation. This may be the reason for the increased protease activity which provided the increased dry matter production. In the reproductive phase, the reduction in the protease activity can be correlated with the reduced utilization of protein. Noggle and Fritz (2002) attributed this reduction to the reduced mobilization of synthesized protein from the source to the sink.

Effective synthesis and utilization of proteins was observed in the lower concentrations. This is evidenced by the increase in protease activity in the lower concentrations. Presence of high concentrations of cations and anions in the effluent might have
created a water stress resulting in the reduced protease activity. This might have affected the protein metabolism. Maranville and Paulson (1972) also reported a similar reduction in protease activity in water stress. According to Somasundaram et al. (1994), the low protease activity in stressed leaves may be attributed to a decrease in the synthesis of fraction I protein (RuBP-carboxylase) the major soluble protein of the leaf. Thus, the changes in the protein metabolism is believed to affect the physical properties of the cell protoplasm and thereby the growth of the plant.

5.4.3. Proline

Accumulation of free proline in the adverse environment is the most striking feature of plants and hence it is considered as a stress indicator. Proline content acts as a storage compound and might be the major source of energy and nitrogen required by the plants for rapid recovery from the adverse environment (Singh et al., 1973). Greenway and Munns (1980) indicated that the adaptive role of proline is related to survival rather than maintenance of growth.

In the present study, an increase in proline content was observed in concentrations above 10% in MO 16 and in MO 19, it was from 20% (Table- 10; Graph- 9). In the vegetative and reproductive phases of growth, concentrations above 30% showed an increase. Greater accumulation of proline was noticed in the reproductive phase over the vegetative phase in both the varieties.
(Table- 33; Figure- 43). Shanta and Karadge (1989), Baruah et al. (1998), Dayal and Goswami (2003) and Azooz et al. (2004) also observed similar results.

The higher concentrations of the effluent, due to the accumulation of dissolved solids, imposed a stress creating a water deficit resulting in the increased accumulation of proline. Moreover, the increased salt content and the toxicity due to the accumulation of ions might have resulted in the increased proline content. Devlin and Witham (1983) reported that the proline act as a storage compound when protein synthesis is reduced as a result of stress. Thus, accumulated proline might be the major source of energy and nitrogen required by the plants for rapid recovery from the adverse environment (Singh et al., 1973).

Proline accumulation may reduce stress-induced cellular acidification or prime oxidative respiration to provide energy needed for recovery. High levels of proline synthesis during stress may maintain NAD(P)+/NAD(P)H ratios at values compatible with metabolism under normal conditions (Hare and Cress, 1997). Sanchez Urdaneta et al. (2005) stated that the increase in proline is resulted from the imbalance in plant metabolism and also this can benefit the stressed plant. This could be possible due to the accumulation of some solutes like proline, which may promote water absorption (Maiti et al., 2004). The increased level of proline in the treated plants may be either due to the breakdown of

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Studies on the effect of distillery effluent on rice (Oryza sativa L.)
protein or due to de novo synthesis (Thompson et al., 1966; Boggess and Stewart, 1980). The increased proline content in the reproductive phase was also observed by Dayal and Goswami (2003). They suggested that the increase is effective in increasing the osmotic potential of the plant.

Enhanced growth in the lower concentrations may result in the reduction of proline content. Proline could serve as a precursor for chlorophyll synthesis mediated by the conversion of glutamate (Rena and Splittstoesser, 1974) and this may result in the alleviation of stress condition. Shanta and Karadge (1989) also made similar conclusions.

5.4.4. Total Phenols

Phenolic compounds have an aromatic ring that contains attached substituent groups like hydroxyl, carboxyl or other non aromatic groups. The biosynthesis of phenolics in plants is linked with glucose molecules in the form of glycosides (Noggle and Fritz, 2002). In higher plants, the production of phenolics is mediated through the acetate mevalonate pathway and also through schikimic acid pathway. Phenolic compounds are essential for the growth and development of plants, and are produced as a response for defending injuries, against pathogens and stresses (Salisbury and Ross, 1995).

In the present study, seedlings showed an increased content in concentrations above 10% in both the varieties (Table-10; Studies on the effect of distillery effluent on rice (Oryza sativa L.)
Figure- 9). In the vegetative and reproductive phases of growth, phenol content showed the maximum in 40 and 50%. More phenol content was observed in the vegetative phase over the reproductive phase (Table- 34; Graph- 44). The results are in unison with the findings of Kadam and Bhosale, (1985), Taghavi and Vora (1994), and Wilson (1998).

The increase in phenol content in the higher concentrations of effluent may be due to the retardation of growth by inhibiting cell division and cell enlargement. Similar conclusion was also made by Taghavi and Vora (1994). Wilson (1998) suggested that the salt induced physiological stress in the higher concentrations of the effluent eventually damages the subcellular organelles, disrupting their specialized functions like photosynthesis, respiration and production of biochemical constituents like phenols. Phenols, the secondary metabolites, affect plant growth in different manner by lowering the activity of hormones, which cause depression in the biosynthesis of the IAA precursor L-tryptophan (Kafeli and Kutacek, 1977).

The phenol content showed a decrease in the lower concentrations where an increased growth was observed. This might be due to the dropping off in the concentration of various chemicals in the effluent (Rajannan and Oblisami, 1979). The presence of growth promoting phenolic compounds might have influenced the increase in plant growth (Bose et al., 1973).
5.5. Oxidizing Enzymes

5.5.1. Catalase

Catalase is one of the key enzymes that are involved in the removal of toxic peroxides. This is mostly universal oxidoreductase that scavenges hydrogen peroxide via a two electron transfer producing water and molecular oxygen (Lin and Kao, 2000). This is an iron containing enzyme that catalyzes the decomposition of hydrogen peroxide and hence it is protective in function (Stiles, 1999). Measurement of catalase activity of a tissue is often accepted as an index of the intensity of the metabolic activity as it has a direct influence on the regulation of metabolic activities in the active growing tissues (Mayer and Anderson, 1959).

In the present study, seedlings showed a gradual decrease along with the increase in concentrations in both the varieties (Table- 13; Figure- 12). In the vegetative phase, concentrations upto 30% showed an increased activity whereas in the reproductive phase, this was upto 20%. Catalase activity was more in the vegetative phase than in the reproductive phase (Table- 38; Figure- 48). The results are in agreement with the findings of Agrawal and Mehrotra (1978), Sinha et al. (1988), Naidu and Raman (1995), Dkhar and Nongkynrih (1996) and Madan Pal et al. (2004). The reduced catalase activity in the reproductive phase can be attributed to the reduced respiratory rate which can be directly correlated with the reduced metabolic processes. A gradual

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Studies on the effect of distillery effluent on rice (Oryza sativa L.)
increase in the catalase activity was observed till panicle initiation and it decreased with time after panicle initiation (Reddy et al., 1985). Kar and Misra (1976) observed a reduction in catalase activity as the growth progressed.

In the present study, increased activity was observed in the lower concentrations while in the higher concentrations the activity was reduced. Catalase directly influences the oxidation-reduction of cytochrome oxidase in mitochondrial respiration (Yokohama, 1956). The enhanced growth and vigorous cell division resulted in the increased respiration rate, which in turn produced high amount of hydrogen peroxide in the cells. To reduce this accumulation, catalase activity increased in plants treated with lower concentrations of effluent. Similar conclusion was made by Naidu and Raman (1995). Rashid and Mukherjee (1991) expressed that, the increased catalase activity is an adaptive trait which seems to be helpful in overcoming any damage to the tissue metabolism by reducing toxic levels of hydrogen peroxide produced during cell metabolism. Kadam et al. (1988) correlated the increase in catalase activity with the increase in chlorophyll content of the plant. Similar correlation can be made in the present study also.

The pollutants may alter the catabolic activities either by damaging the tissue or by disturbing the metabolites. Effluent stress reduced the plant growth in the higher concentrations and this resulted in the reduction of catalase activity. Bharadwaj (1964)
reported that the reduced catalase activity was due to the lack of substrate as the respiratory rate was low. Mac Rae and Ferguson (1985) regarded the decline in catalase activity as a general response to many stresses and it is apparently due to the inhibition or change in the assembly of enzyme subunits. Naidu and Raman (1995) attributed the reduction in catalase activity in the higher concentrations to the decrement of chlorophyll content. Madan Pal et al. (2004) suggested that the decrease in catalase activity may be due to the accumulation of hydrogen peroxide which is associated with the tolerance mechanism through signal transduction. Thus, the lowered catalase activity can be attributed to the reduced respiratory rate due to the accumulation of hydrogen peroxide.

5.5.2. Peroxidase

Peroxidases are enzymes, which are located in cytosol, vacuole, cell wall and extracellular spaces, utilize hydrogen peroxide in the oxidation of various inorganic and organic substances (Asada, 1994). Peroxidases catalyze the dehydrogenation of a large number of organic compounds such as phenols, aromatic amines, hydroquinones etc (Sadasivam and Manickam, 1996). The role of peroxidase as stress enzyme in plants has been generally accepted (Gasper et al., 1991) and these are the first enzymes to alter the activity under the stress. They

Studies on the effect of distillery effluent on rice (Oryza sativa L.)
play a regulatory role in the phytohormone metabolism, because they take part in the catabolism of IAA (Gazaryan et al., 1996)

In the present study, seedlings after 96 hours of germination showed a gradual increase in peroxidase activity with the increase in concentrations with the maximum in 30% in both the varieties (Table-13; Figure-12). In the vegetative and reproductive phases also, a similar trend was observed with the maximum activity in 40% and 50% in both the varieties. Reproductive phase showed more peroxidase activity than the vegetative phase (Table-46; Graph-47). The results are in agreement with the findings of Levitt (1972), Behera and Misra (1985), Sinha et al. (1988), Mishra et al. (1993) and Singh et al. (2003).

Peroxidase is known to deplete the pool of free radicals from accumulating to toxic levels during stress situations and the role as scavenging system to maintain the metabolic functions of the cells to ward off the adverse environment (Vasantha and Rao, 2003). The increased peroxidase activity along with the increase in effluent concentration in the seedlings could be possibly due to the destruction of cellular membrane and the concomitant release of previous immobilized proteins (Farkas et al., 1964) or due to the cessation of cell elongation (Gardiner and Cleland, 1974). Levitt (1972) suggested that the increased peroxidase activity in the seedlings might be considered as the indicator of physiological stress.
In the vegetative and reproductive phases, the lower concentrations upto 20% showed a reduction in the peroxidase activity and thereafter an increase. The enhanced plant growth and increased amount of chlorophyll pigments in the lower concentrations reduced the peroxidase activity. This can be evidenced by the reduced phenol content also, where peroxidase brings about the oxidation of phenolic compounds (Styles, 1999). In the higher concentrations, the increased peroxidase activity was observed. The increased salt content in the higher concentrations might have induced a water stress by the increase of osmotic potential leading to the generation of reactive oxygen species including hydrogen peroxide and this play an important role in inhibiting the plant growth (Lee et al., 2001). Kalir and Poljakoff Mayber (1984) attributed the increase in peroxidase may be due to the increased levels of reactive oxygen species (ROS) including $O_2^-$. Shim et al. (2003) reported that the increase in hydrogen peroxide in plant cell under stress may induce the activity of enzymes to overcome the stress. Thus, increased peroxidase activity effects through the oxidative metabolism can be directly related to the reduction in the physiological and biochemical processes.

**Isozyme**

Plant peroxidase is a stable enzyme occurring in upto twenty isozymes soluble in aqueous medium and present in the whole cell (Yip, 1964). Due to its implication in various physiological and
biochemical course of actions, the occurrence may vary in quality and quantity (Shannon, 1969). The role of peroxidases as stress enzymes in plants has been widely accepted and it has been shown that the peroxidase activity can be used as a potential biomarker (Radotic et al., 2000).

In the present study, even though peroxidase exhibited the difference in intensity of bands between the treatments, there was no variation in the isozyme pattern between the treated and untreated plants in both the varieties (Plates – 8 & 9). Peroxidase may be a part of the defense system and the peculiar feature of peroxidase is their redundancy that several isoforms can ensure the same reactions (Hammerschmidt et al., 1982).

5.6. Photosynthetic pigments

The chlorophylls are the essential components for photosynthesis and occur in chloroplasts as green pigments in all photosynthetic plant tissues. In higher plants chlorophyll-a and chlorophyll-b are the major components of chlorophyll pigment (Taiz and Zeiger, 2003).

In the present study, pigment content like total chlorophyll, chlorophyll-a and chlorophyll-b were increased in the concentrations upto 30% and 40% in MO 16 and in MO 19 respectively. Increased pigment content was observed in the reproductive phase over the vegetative phase (Tables- 40-42, Figures- 50-52). The results are in corroboration with the findings

Studies on the effect of distillery effluent on rice (Oryza sativa L.)
Chlorophyll content of a plant is directly proportional to the photosynthetic efficiency and leaf area (Ashraf and Ali, 1998, Taiz and Zeiger, 2003). Increased pigment content in the lower concentrations might be due to the enhanced plant growth. This is further corroborated by the increased availability and uptake of nutrients that influence the chlorophyll synthesis (Sinha et al., 1988). Reduction in the photosynthetic pigments in higher concentrations may be attributed to the presence of inhibitory effect of toxicants in the effluent (Singh et al., 2006). Increased salt concentration in the effluent might have induced a water stress and this resulted in the shrinkage of chloroplast and disruption of thylakoid structure (Mukherji and Ghosh, 2006). Increased chlorophyll content in the reproductive phase was also observed by Gomathi and Oblisami (1992). They attributed this increase to the enhanced nutrient availability of the effluent.

The reduced plant growth also contributes to the reduction of the photosynthetic pigments (Saxena and Jabeen, 1989). Vasanthy and Lakshmanaperumalsamy (1998) suggested that the reduction in chlorophyll may be due to the increased concentration of TDS, TSS, chloride, sulphate, nitrate etc which destabilize the chloroplast pigment. Rani and Janardhan (1988) opined that, high

Studies on the effect of distillery effluent on rice (*Oryza sativa* L.)
amount of chlorides in the effluent might have reduced the chlorophyll pigments. The decrement in the present study can also be correlated with the above statement. Izawa (1977) suggested that the inhibition of chlorophyll may be due to the induced inhibition of electron transport system in PS-II. Thus, the reduction in chlorophyll content in the higher concentrations in the present study might have led to the reduction in the carbon assimilation resulting in the reduced growth.

5.7. Nutrient Uptake

Green plants require a varied amount of nutrients for the metabolic activities but a marked difference is observed in its accumulation and utilization and these vary from species to species. Nitrogen, phosphorus and potassium are the cardinal elements that the plants absorb to perform various metabolic activities.

In the present study, concentrations upto 30% showed a profound increase in nitrogen, phosphorus and potassium content over the higher concentrations in both the varieties. Nitrogen and phosphorus content was more in the vegetative phase but potassium content showed a varying effect (Tables- 43 - 45, Figures– 53 - 55). The results of the present study are corroborative with that of Chattergee et al. (2003), Medhi et al. (2005), Rajeswari et al. (2005) and Sivaraman and Thamizhiniyan (2005).
The physicochemical analysis of the effluent showed that it is with a high load of nitrogen, phosphorus and potassium. The increased levels of nitrogen, phosphorus and potassium in the plants irrigated with lower concentrations of effluent might have increased the uptake of these nutrients resulting in the increased nutrient content in the tissues leading to the better growth. The increased nutrient content can also be attributed to the availability of these elements in their optimum quantities and the favorable osmoticum prevailing in the effluent for their absorption. Salisbury and Ross (1995) correlated the uptake of solutes with the lowered water potential and high turgor. The increased uptake might also be due to the osmotic adjustment in the cells (Taiz and Zeiger, 2003). Rajeswari et al. (2005) suggested that the hike in nutrient uptake in the diluted effluent might be due to the reduction of the constituents to the beneficial levels.

The reduction in nutrient content in the higher concentrations may be due to the excess supply of mineral ions, which can cause a negative impact on nutrient uptake and also in reduction of growth. The treatment with higher concentrations of effluent might have also changed the electrical conductivity and pH, resulting in the low uptake of nutrients. The increased osmotic potential of the rooting medium created a water stress resulting in the reduced uptake of nutrients (Greenway and Munns, 1980). Here also, the increased salt concentrations of the effluent might

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Studies on the effect of distillery effluent on rice (*Oryza sativa* L.)
have created a similar stress resulting in the reduced uptake of nutrients. Besides these, due to the accumulation of excess ion concentration in the effluent might also have created the ion toxicity resulting in the reduced uptake of nutrients (Medhi et al., 2005). Gratten and Grieve (1994) opined that the increased salt concentrations may cause physiological inactivation of the particular nutrient resulting in the increase in the internal requirement for that element. Tripathi (1978) attributed the reduction in nutrient content in the higher concentrations to the hindrance in the uptake and metabolism of the nutrients due to excessive dissolved salts and osmotic imbalance. These facts indicate that, the increased nutrient content of the effluent might have caused a reduction in the uptake of nutrients, which might have affected the growth and metabolism and eventually resulting in the reduced yield.

Studies on the effect of distillery effluent on rice (*Oryza sativa* L.)