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Research Article

Evaluation of Jatropha Deoiled Cake Based Composts for Sustain Growth of Rice *(Oryza Sativa* L.*)* Under Drought Conditions

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Abstract

Drought stress is a major yield destabilizing factor affecting a large area under agriculture. However, compost plays a vital role in tolerance of drought stress as it is a prerequisite for favorable soil properties such as aggregate stability and porosity beside supply of nutrients. In present study two different composts were used: (i), a mixture of jatropha deoiled cake, rice straw and cow dung (JRsC) (ii) mixture of jatropha deoiled cake, neem cake, mushroom spent and *Cuscuta reflexa* (JNMsC). Pot culture experiment was setup under glasshouse conditions to determine the drought stress mitigating effect of compost on rice *(Oryza sativa* L.*)* plants. Seedlings of 25-day-old rice (Sahbhagi Dhan var.) were transplanted to plastic pots filled with soil and compost (20%). Irrigation was stopped to induce drought stress before flowering stages. Photosynthetic pigments, nitrate reductase activity, proline and activities of antioxidant enzyme (Superoxide dismutase) were estimated from leaves of both control and compost treated plants. Drought stress caused significant decline in photosynthetic pigments and nitrate reductase activity, while composts under proper irrigation showed significant increment in the content of both the parameters over control. There was a significant enhancement in proline and superoxide dismutase production in control plants under drought stress when compared to irrigated control as well as compost amended drought stressed plants. Results depicts that compost can be used as a precursor to increase productivity and sustainability of crops.
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Keywords: Compost, Drought, Chlorophyll, SOD, NRA, Proline, *Oryza sativa*.

Introduction

Rice (*Oryza sativa* L.) has been the important primary cereal and largest irrigated crop for more than two third of the world's population (Dowling et al. 1998; Roel et al. 1999). More than 75% of the world's rice supply comes from irrigated land of Asia; therefore, the present and future food security depends largely on the rice production system. This system requires huge amount of water for production (Tabbal et al. 2002). The available amount of water for irrigation, however, is increasingly getting scarce (Pirdashti et al. 2009). The severity of drought is unpredictable as it depends on many factors such as occurrence and distribution of rainfall, evaporative demands and moisture storing capacity of soils (Wery et al. 1994). Environmental factors that impose water-deficit stress, such as drought, salinity and temperature extremes, place major limits on plant productivity (Boyer, 1982). Rice is often considered as one of the most drought sensitive cereal crop. Water deficit mainly occurs during the growing season, and the intensity of such stress depends on the time span and frequency of water deficit. In country like India, drought is a serious constraint on the productivity of main agricultural crops such as wheat and rice. It is known that drought can affects plant physiology, inhibits root growth, dry matter production and also reduces the yield and its components (Agnew and Warren, 1996; Tas and

Tas, 2007). Tolerance to abiotic stresses is very complex, due to the intricate of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development (Razmjoo et al. 2008). High yield potential under drought stress is the target of crop breeding. In many cases, high yield potential can contribute to yield in moderate stress environment (Blum, 1996).

The use of microbiologically rich compost for agricultural production could alter these relationships and help in increasing crop productivity in both ways qualitatively and quantitatively. Number of evidences indicates that single microorganism inoculation do not provide a persistent solution for the long-term sustainable growth of plants, therefore, a consortium of microorganisms is needed for creating the beneficial rhizosphere (Adani et al. 1997). Although the potential benefits of compost and rhizosphere microorganisms for decreasing plant stress caused by abiotic and biotic factors have been shown in various case studies (Gomez 1998), however, there is little knowledge about the importance of this phenomenon in plant rhizosphere ecology.

Water is one of the most important ecological factors determining crop growth and development; water deficit plays a very important role in inhibiting the yields of crops (Zhu, 2002). Water-limited crop production depends on the intensity and on the pattern of drought, which vary from year to year. It is another important and significant factor restricting plant growth and crop productivity in the majority of agricultural fields of the world (Tas and Tas 2007). It inhibits the photosynthesis of plants, causes changes in chlorophyll contents and components and damage to the photosynthetic apparatus (Nayyar and Gupta 2006). In addition, it inhibits the photochemical activities and decreases the activities of enzymes in the Calvin Cycle in photosynthesis (Monakhova and Chernyadev 2002). High application of compost may be a very useful tool for ameliorating severely drought affected areas through the establishment of plant cover.

Green revolution has witnessed higher productivity, however, has caused reduction in soil health. Organic matter reserves have declined and they are becoming the limiting factor especially under tropical situation. Utilization of compost is a relatively new biological way of decreasing abiotic stress in plant production. Composting has been defined as intense microbial activity leading to decomposition of most biodegradable materials, usually mixtures of organic materials, which results in organic residue stability (Adani et al. 1997). It can be considered as a soil conditioner that contributes to soil fertility, structure, porosity, organic matter, water holding capacity and disease suppression (Itävaara et al. 1997; Biala1 et al. 2008). Thus, the use of compost has the potential to decrease abiotic stress of cultivated plants. Jatropha (*Jatropha curcas* L.) is being used as biofuel plants, however, the deoiled cake is unusable because of the forbol esters though it is a very rich source of protein. Use of compost from Jadropha deoiled cake mixed with other substrates such as animal dungs, crop residues etc could be useful. It has high nitrogen content and can be used as valuable organic manure. In addition, it shows insecticidal properties and controls various pests. Therefore, present study was designed to determine the effect of role of Jatropha deoiled cake composts on reducing drought stress.

Materials and Methods

Compost

In present study, two different composts were used: (i) mixture of jatropha deoiled cake, rice straw and cow dung (JRsC,) supplied by The Energy and Resource Institute (TERI), Delhi, India (ii) mixture of jatropha deoiled cake, neem cake, mushroom spent and amarbel (*Cuscuta reflexa)* (JNMsC) prepared at the G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India, by using Berkley rapid composting, shredding and frequent turning method (Raabe, 2001). Jatropha deoiled cake (50%), neem cake (25%), mushroom spent (25%) and *Cuscuta reflexa* (10g) were mixed together and filled in the steel pit with 80% moisture at the beginning. The piles were periodically turned for aeration and water was added to maintain the moisture content 50-60%. The temperature of the composting materials was kept to 50-60°C for several weeks. As the activity slows down the temperature also drops gradually and reaches at ambient air temperature finally. After 90 days of composting, the finished compost was collected from the respective pit and air dried.

Experimental Design

The experiment was conducted under glasshouse conditions. Supplementary light was provided by cool white lamps, 400 µE m^{-2} s^{-1}, 400-700 nm, with a 16/8 h day/night cycle at $\pm$ 27^{o}C and 60% relative humidity. Two different composts; JRsC and JNMsC, were used @ 20% of soil. Seedlings of 25-day-old rice (Sahbhagi Dhan) were transplanted to plastic pots (0.5l cap) filled with a mixture of soil and compost. Water holding capacity of soil was determined before adding compost. There were two conditions; irrigated and drought induction. The experiment was conducted in factorial completely randomized design with four replications of each treatment. The pots were irrigated regularly with equal amount of water on the basis of determined water holding capacity. Irrigation was interrupted to induce drought stress at flowering stage in half of the pots of each treatment, whereas, other half pots were regularly irrigated as per water holding capacity. The plants were harvested at three different times; 6, 10 and 25 days after inducing the stress.

Estimation of Photosynthetic Pigments (Chlorophyll and Carotenoids)

Five hundred mg of leaf material was collected from the second and third nodes of the shoot tip. Extraction of pigments was achieved with 80 % acetone in cold room. Extract was centrifuged at 5000 rpm for 10 minutes. Extraction was done twice. The supernatant containing chlorophyll and carotenoids was made up to 3 ml. Absorption of clear supernatant was determined with double beam spectrophotometer (Ray Leigh, UV-2601) at 440 nm for carotenoids and at 645, 652 nm and 663 nm for chlorophylls. . A solution of 80% acetone was used as a blank. The Chla, Chlb, total chlorophyll and carotenoids (mg g^{-1} FW) concentrations in the leaf tissues were calculated according to the method of Tuba (1987).

Nitrate reductase activity

The activity of nitrate reductase in plants was assayed *in vivo* following the method of Srivastava (1975). Freshly harvested plant leaves (500mg) were taken in black vials of 20 ml, containing 8.0 ml of 0.1 M sodium phosphate buffer (pH 7.4), 1.0 ml of 0.2 M KNO_3 and 1.0 ml of 25% propanol. These vials were sealed and incubated in dark for 30 minutes at 30^0C. Nitrite released in incubation mixture due to enzymatic activity was measured by colour development. For this, 1.0 ml 1% sulphanilamide in 1 N HCl and 1.0 ml 0.2 % N-(1-naphthyl) ethylenediamine-dihydrochloride (NED) was added in 1.0 ml aliquot from the incubation mixture. After 20 minutes, absorbance was read by spectrophotometer (Ray Leigh, UV-2601) at 540 nm and was calculated as millimole NO_2^- hr^{-1} g^{-1} FW.

Proline content

Proline content was determined following the method of Bates et al. (1973). The young expanded leaves (200mg) were homogenized in 4 ml of sulfosalicylic acid (3%) and centrifuged at 10,000g for 30 min. Two ml of extract supernatant was taken in a test tube and to it glacial acetic acid (2ml) and 2 ninhydrin reagent (2ml) was added. The reaction mixture was boiled in water bath at 100^0C for 30min. After cooling the reaction mixture, 4 ml toluene was added and vortex for 30sec. The upper phase containing proline was measured with spectrophotometer (Ray Leigh, UV-2601) at 520 nm using toluene as a blank. Proline content (µmol g^{-1} fr. wt.) was quantified using the ninhydrin acid reagent by using L-proline as a standard.

Superoxide Dismutase

Superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) (Giannopolitis and Ries, 1977). The reaction mixture (1.5 ml) contained 50mM phosphate buffer (pH=7.8), 0.1µM EDTA, 13mM methionine, 75µM NBT, 2µM riboflavin and 50µl enzyme extract. Riboflavin was added last and tubes were shaken and illuminated with a two 20W fluorescent tubes. The reaction was allowed to proceed for 20 min. after which the lights were switched off and tubes were covered with a black cloth. Absorbance of the reaction mixture was read at 560nm. One unit of SOD activity (U) was defined as the amount of enzyme required to cause 50% inhibition of NBT photo reduction rate and the results expressed as U mg^{-1} of fresh weight.

Data analysis

The statistical evaluation of complete data was done and all analyses were performed based on four replicate pots. Data were subjected to one-way analysis of variance (ANOVA) with different physiological and biochemical parameters (JMP 5.0, SAS Institute, Cary, NC, USA) to analyze the effect of each treatment separately. The treatment means were separated using Tukey HSD at 0.05% probability level

Results

Photosynthetic pigments

A significant decline in Chl 'a' content was observed in control sample of stress treatment over to JRsC samples of both irrigated and stress treatment, after 6 days (fig. 1). However, Chl 'b' of control under stress condition decrease significantly. Chl't' (total chlorophyll) and carotenoids content of the same was significantly different with all other samples of irrigated and stress treatment.

After 10 and 25 days harvesting all results of Chl ‘a’, Chl ‘t’ (total chlorophyll) and carotenoids content showed significant different in comparison to control of stress treatment (fig. 2 and 3). However, in 10 days harvested samples Chl ‘b’ was observed non-significant only with control (irrigated) and JRsC (stress), whereas, in 25 days harvested samples, same was found non-significant only with JNMsC under stress condition.

Proline content

In samples harvested after 6 and 10 days, proline content of control (stress) increased significantly over to JRsC and JNMsC compost (stress). On the contrary, control (irrigated) showed significant low values over JRsC and JNMsC compost (irrigated) (fig. 4 and 5). However, incase of 25 days harvesting, control under irrigated conditions showed the similar results as that of 6 and 10 days. Furthermore, under stress, inverse results of proline content were obtained, i.e. JRsC and JNMsC amended samples were found significantly increased in comparison to control under stress conditions (fig. 6).

Nitrate reductase activity

Over all NR activity was significantly reduced in control of both the treatments (stress and irrigated) over to JRsC and JNMsC (fig. 7 - 9). However, in 6 days harvested samples, non-significant results were observed between JRsC and JNMsC of stress and irrigated treatment both, while in 10 days harvested samples, non-significant values were observed between JRsC (irrigated) and JNMsC (stress). Furthermore, after 25 days of harvesting, significant decrease was observed in values of JRsC and JNMsC under stress over to irrigated one.

Superoxide dismutase

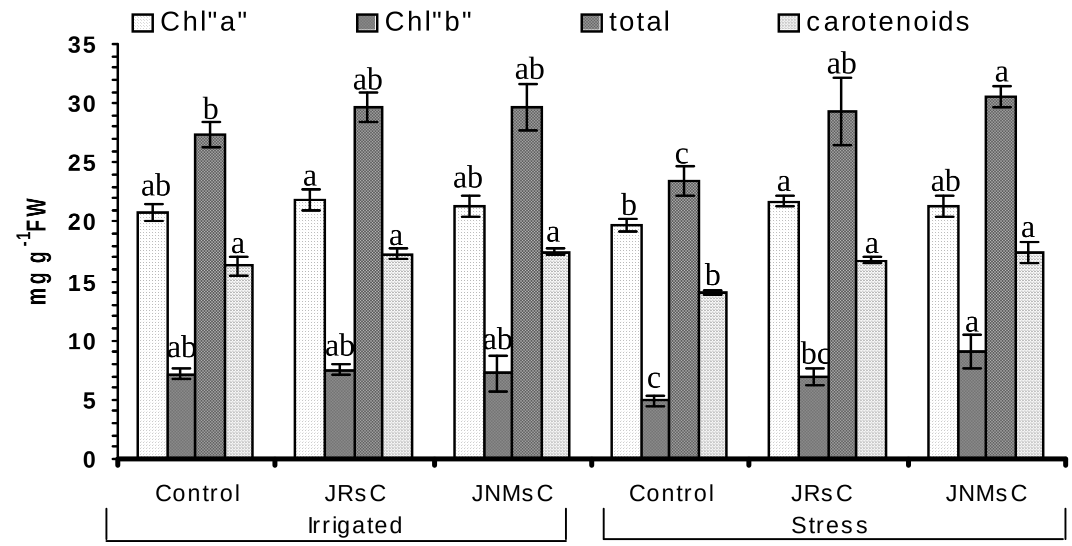
After 6 days, the control of stress and irrigated rice were non-significant with each other, whereas, same were significantly higher in compost (JRsC and JNMsC) amended treatment (fig. 10). However, in 10 and 25 days harvested samples, control samples under stress condition resulted in significant increase over all other values (fig. 11 and 12). In addition, JRsC and JNMsC under stress conditions were found non-significant with irrigated samples.

Discussion

Drought stress caused significant declines in chlorophyll and carotenoids content. This variation in photosynthetic pigments level during stress depends on the duration and severity of drought (Anjum et al. 2003; Farooq et al. 2009). Under stress stomata get blocked, which leads to a decrease in absorbing CO_2, however, on the other hand the plants consume a lot of energy to absorb water. These reasons may cause a reduction in producing photosynthetic matter. Results also depict maximum fluctuation in chlorophyll ‘b’ under stress conditions. It was also reported earlier that chlorophyll ‘b’ is more sensitive than chlorophyll ‘a’ and carotenoids under stress conditions (Netondo *et al.* 2004). The possible reason for this may be due to the structural variation and localization of chlorophyll ‘b’ in antennae complex (Taiz and Zeiger, 2003). Furthermore, stress condition could disrupt components of plant’s photosynthetic system, such as membrane integrity and further decrease photosynthetic capacity (Demmig-Adams and Adams, 1992), which could be probably the reason for decrease in photosynthetic components in control of stress treatment. However, the higher total chlorophyll concentration of plant leaves was observed in composts (JRsC and JNMsC) over to control as they had a potential for improving plant growth. Compost acts as a soil conditioner and contributes in structure, porosity, and water holding capacity (Itävaara et al. 1997). They also help in increasing in soil fertility and organic matter, which leads in making a strong and ramified root system. Such root systems may implicate in the drought tolerance and high biomass production, due to this ability they extract more water from soil and transport to aboveground parts for photosynthesis.

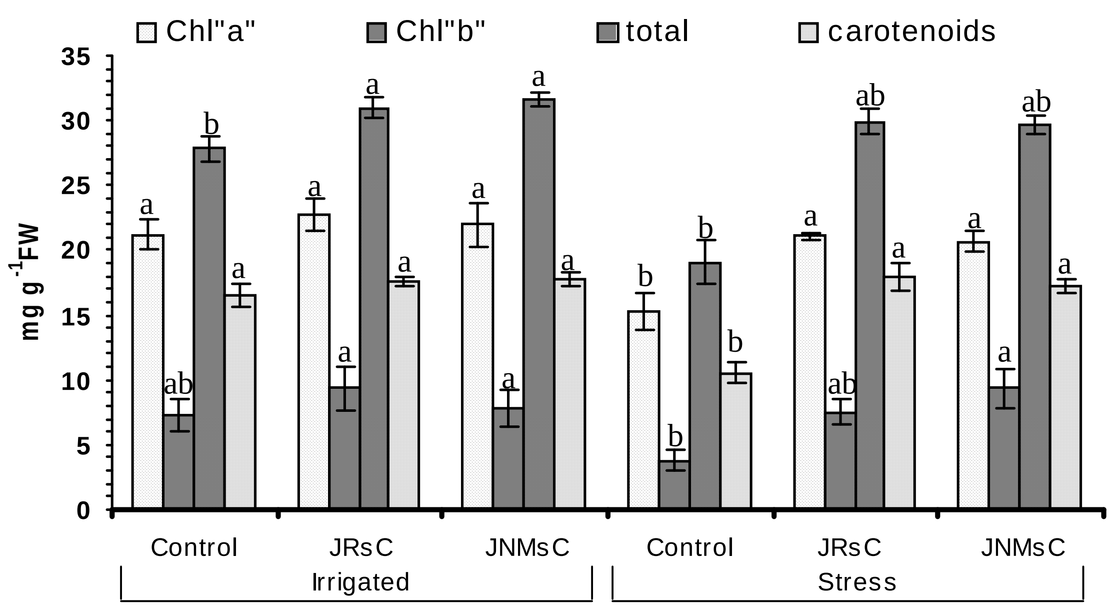
Proline increases proportionately faster than other amino acids in stressed conditions; therefore, has been generally selected to evaluate the environmental stress studies. Plant cells accumulate proline as osmoprotectant solute to adjust osmotic stability, control redox potentials and prevent damage during drought stress. Reduce concentration of proline in composts (JRsC and JNMsC) of stress treatment may be due to low injury in compost treated plants as a result of drought avoidance, while high concentration of water stress (as found in control) causes impaired plant health, which results in rapid ammonium accumulation and results in detoxification process in which excess ammonium in the cells, is removed. This mechanism results in the higher accumulation of low molecular weight water soluble, nitrogen containing metabolites, like proline in their cells for osmotic adjustment (Jain et al. 2001). However, the present study is amply documented with the results of Lutts et al. (1996). They reported that abiotic tolerant cultivated rice accumulates less free proline than stress sensitive one. Nevertheless, increased proline content

Fig.1. Effects of composts on photosynthetic pigment composition in rice (*Oryza sativa* L.) under drought stress of 6 days.



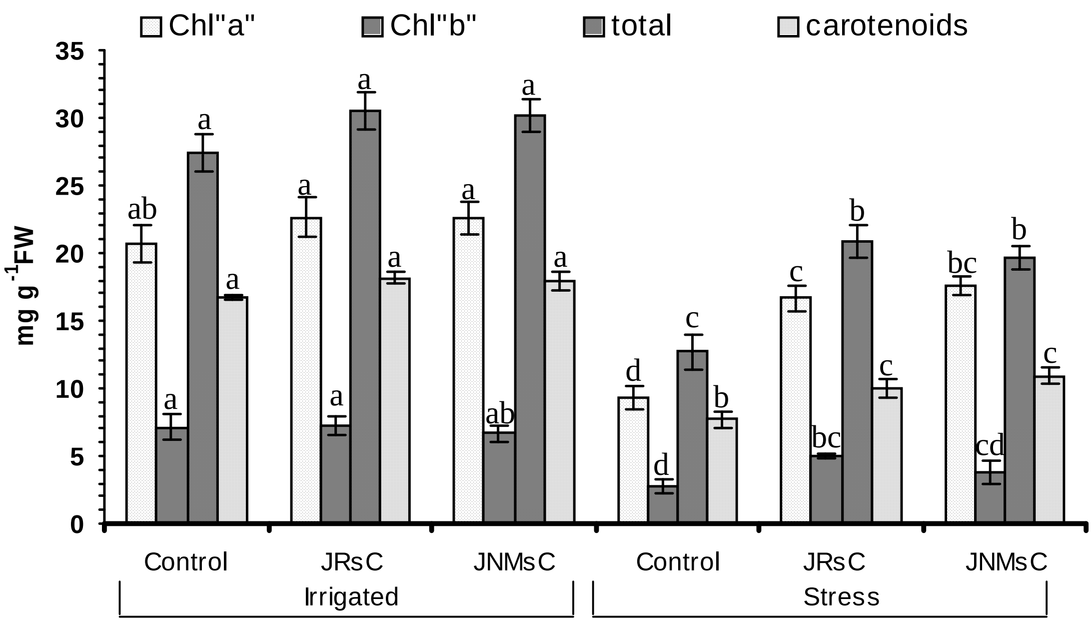
* Chlorophyll a (Chl "a"), Chlorophyll b (Chl "b"), Total Chlorophyll (total), Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)
*Level not connected by same letter are significantly different

Fig.2. Effects of composts on photosynthetic pigment composition in rice (*Oryza sativa* L.) under drought stress of 10 days.



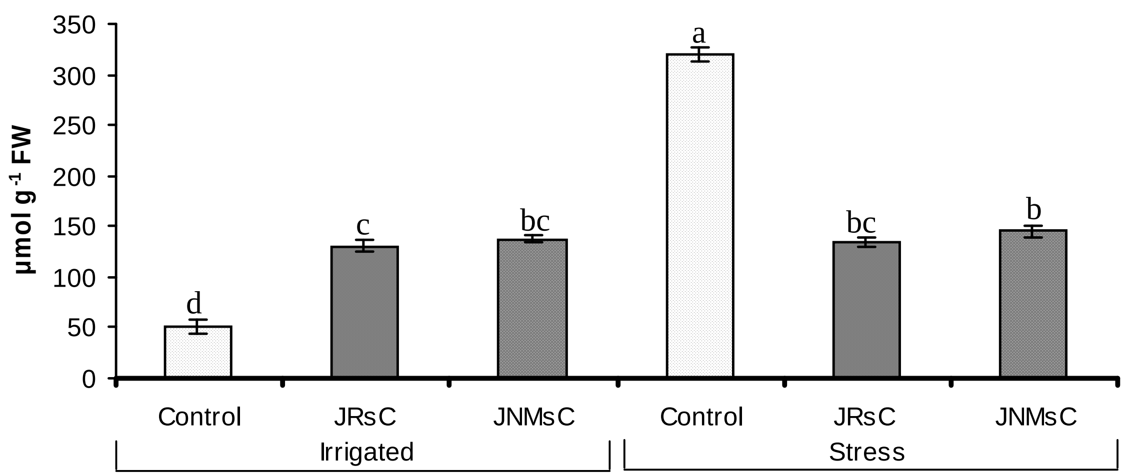
* Chlorophyll a (Chl "a"), Chlorophyll b (Chl "b"), Total Chlorophyll (total), Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)
*Level not connected by same letter are significantly different

Fig.3. Effects of composts on photosynthetic pigment composition in rice (*Oryza sativa* L.) under drought stress of 25 days.



* Chlorophyll a (Chl “a”), Chlorophyll b (Chl “b”), Total Chlorophyll (total), Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)
*Level not connected by same letter are significantly different

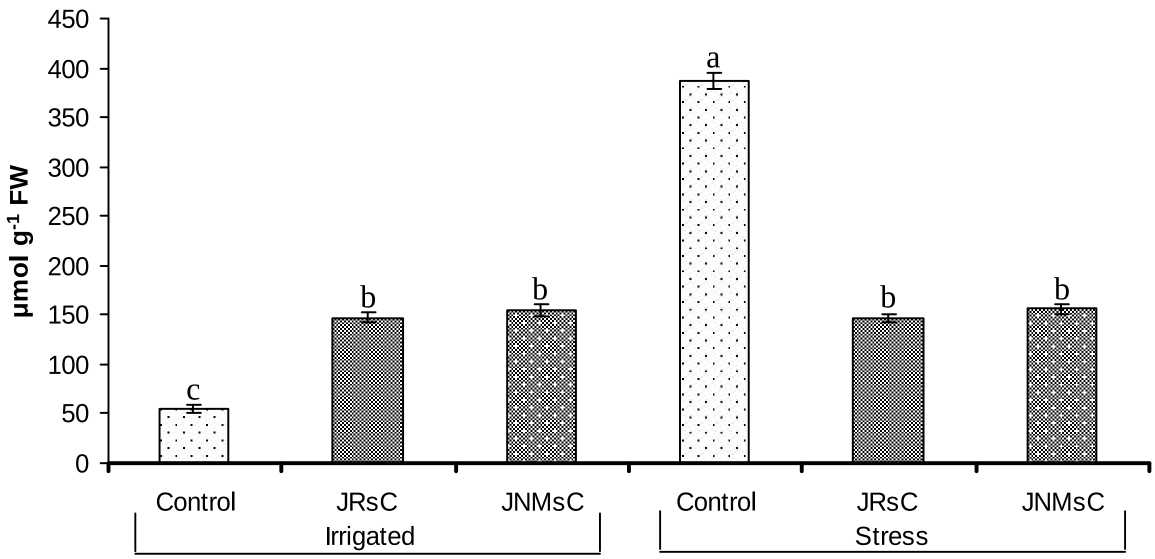
Fig.4. Effects of composts on proline content in rice (*Oryza sativa* L.) under drought stress of 6 days.



*Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)

*Level not connected by same letter are significantly different

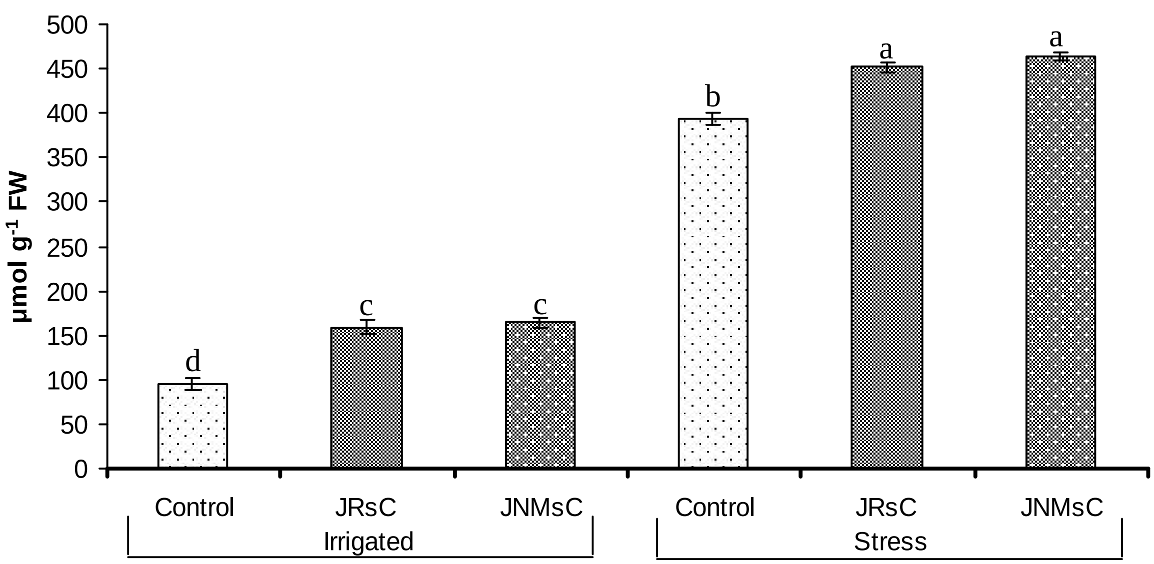
Fig.5. Effects of composts on proline content in rice (*Oryza sativa* L.) under drought stress of 10 days.



*Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)

*Level not connected by same letter are significantly different

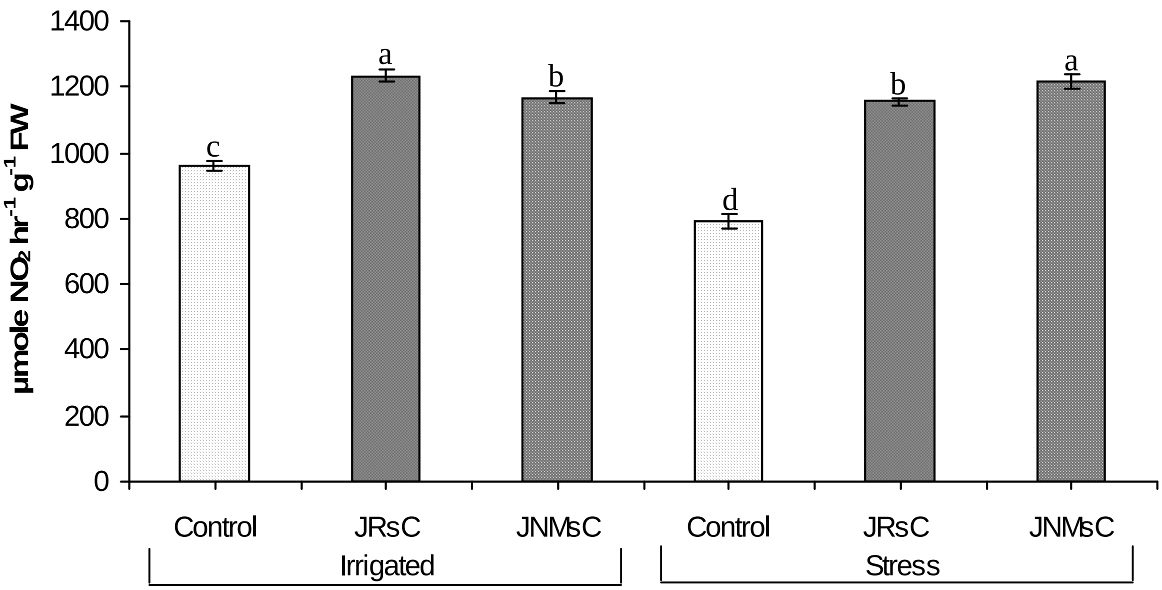
Fig.6. Effects of composts on proline content in rice (*Oryza sativa* L.) under drought stress of 25 days.



*Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)

*Level not connected by same letter are significantly different

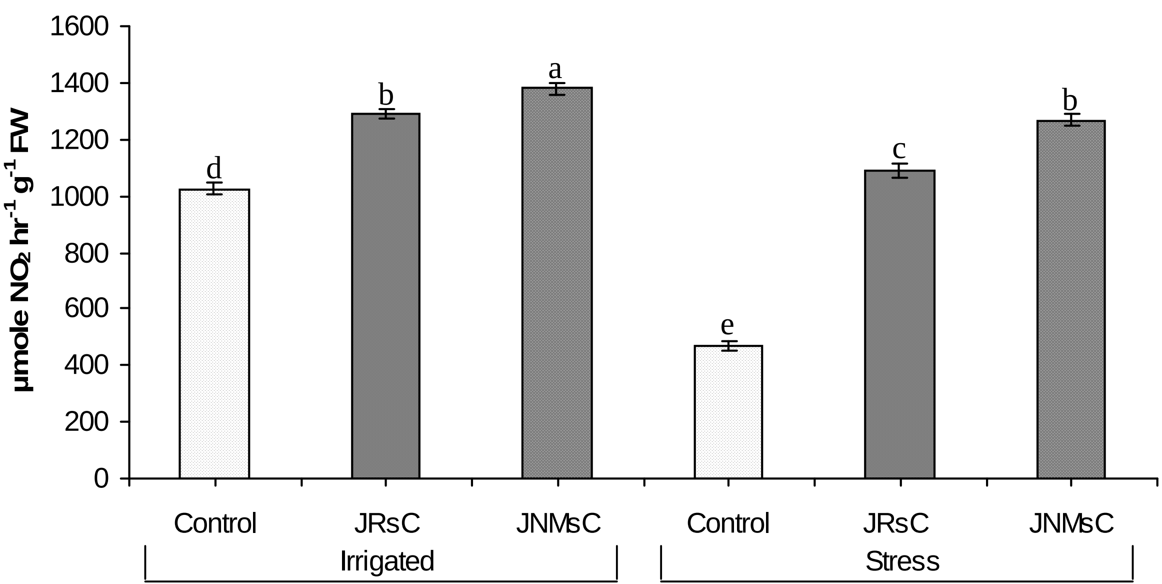
Fig.7. Effects of composts on nitrate reductase in rice (*Oryza sativa* L.) under drought stress of 6 days.



*Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)

*Level not connected by same letter are significantly different

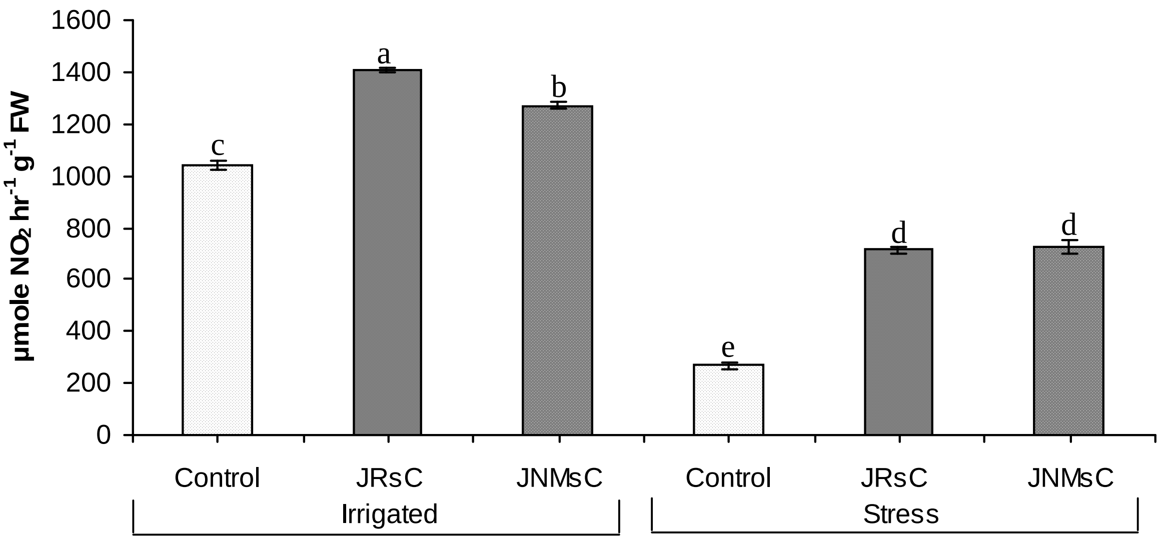
Fig.8. Effects of composts on nitrate reductase in rice (*Oryza sativa* L.) under drought stress of 10 days.



*Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)

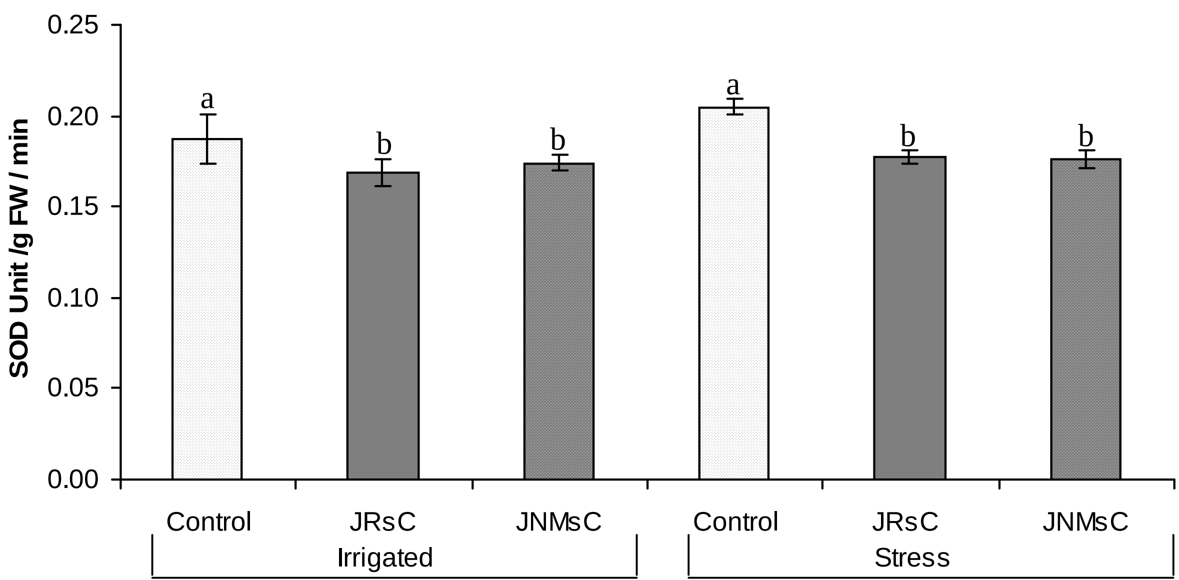
*Level not connected by same letter are significantly different

Fig.9. Effects of composts on nitrate reductase in rice (*Oryza sativa* L.) under drought stress of 25 days.



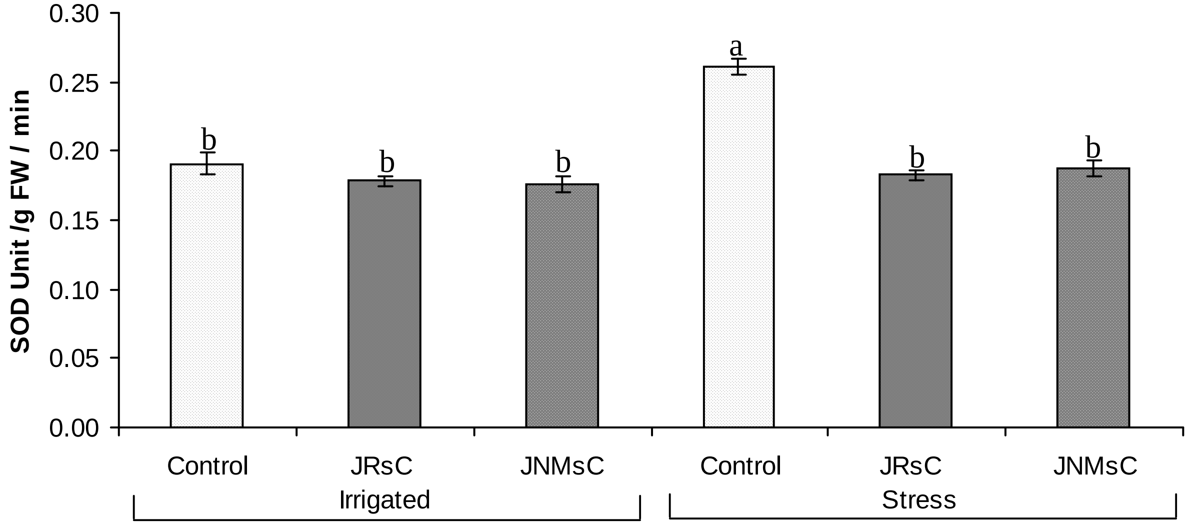
*Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)
*Level not connected by same letter are significantly different

Fig.10. Effects of composts on superoxide dismutase in rice (*Oryza sativa* L.) under drought stress of 6 days.



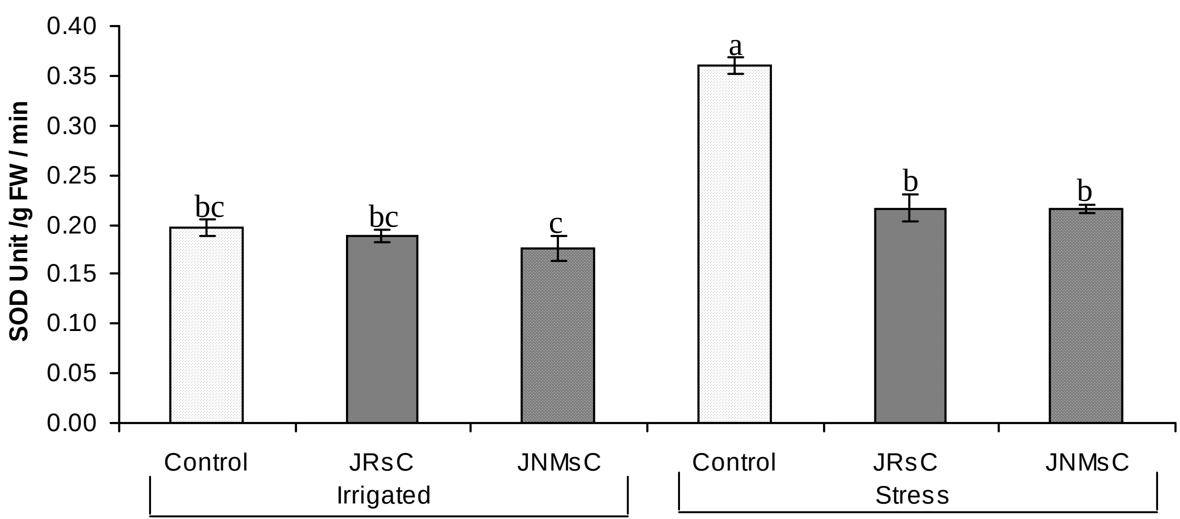
*Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)
*Level not connected by same letter are significantly different

Fig.11. Effects of composts on superoxide dismutase in rice (*Oryza sativa* L.) under drought stress of 10 days.



*Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)
*Level not connected by same letter are significantly different

Fig.12. Effects of composts on superoxide dismutase in rice (*Oryza sativa* L.) under drought stress of 25 days.



*Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)
*Level not connected by same letter are significantly different

was observed in irrigated rice cultivars amended with compost (JRsC and JNMsC) over control. This may be probably due to high microbial activity in compost which results in rapid nitrification of ammonium mineralized from organic material. Therefore, nitrate gets converted into ammonium, which found to be responsible for higher proline content (Ruiz-Lozano and Azcon, 1996).

Compost treated plant often show either decrease or increase accumulation of amino acid and nitrogenous compounds in addition to greater enzymatic activity under abiotic stress condition. The effect of stress on NR activity was observed inhibitory. Nitrate reductase enzyme plays a central role in plant primary metabolism and exhibits complex regulation mechanism for its catalytic activity (Viégas and Silveira, 2002). The process of N assimilation starts with the reduction of NO_3 to NO_2 by nitrate reductase. This step usually serves as a rate limiting factor in the N assimilation process and drastically slowed down by drought stress (Ruiz-Lozano, 2003). However, compost improved nutritional status and nitrogen assimilation rate of water stressed plants. In present study, NR activity increases in compost over to control in irrigated samples. It indicated that the activity of enzyme nitrate reductase is inhibited in the tissue by the application of stress condition. Nevertheless, NR activity of compost under stress conditions increases over to control.

Plants also have a complex antioxidant system to avoid the harmful effect of Reactive oxygen species (ROS) (Arnon and Sairam, 2002; Sharma and Dubey, 2005; Gambarova and Gins, 2008). However, detoxification of excess ROS is essential as the high concentration of ROS disrupt the normal physiological and cellular functions (Gille and Sigler 1995). Furthermore, it is also documented that, greater SOD is associated with decreased damage to photosynthetic apparatus (Golkar et al. 2009). Results show maximum SOD activity in control of stress over compost containing plants, which means that SOD activity increases whenever plant experience stress. This may be due to ROS scavenging mechanism of plant include which act as defense enzymes and protect plant from oxygen toxicity caused by the abiotic stress (Lin and Kao, 1998).

Conclusion

India has been experiencing changes in climate as elsewhere in the world as exemplified by unpredictable monsoon, rains, floods and extreme temperature etc. Drought is the most serious natural disaster greatly affects on crops growth and development, hasten maturity, and reduce soil moisture. Therefore, improvements against this destabilizing factor is essential for maintaining local and global food security, particularly in India where climate change is predicted to significantly increase the intensity and frequency of drought stress. Jadropha deoiled cake based compost plays a vital role in avoidance and tolerance of above discussed stress. It is a valuable green manure obtained as a byproduct after oil extraction, however, it can not be used directly because of its toxic properties but, when it mixed with inert carrier or organic manure it becomes a soil enricher and helps in plant growth. The biologically active compounds from compost have the potential to improve the drought tolerance of crops. Further, study in this direction may highlight a better understanding of the mechanisms adopted by plants in response to environmental stress factors and how to survive under such conditions. Hence, compost can be used as a precursor to increase productivity and sustainability of crops.

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