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Original Research Article

Role of physic nut (*Jatropha curcas*) Deoiled Cake Based Composts in Sustainable Growth of *Brassica campestris* Under Water Deficit: An eco-friendly approach

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Abstract: Water deficit conditions constitute the most significant factors leading to substantial and unpredictable decrease in crop yield. In order to redress the constraints and also increase crop productivity in a sustainable way a study was, conducted on utilization of *Jatropha curcas* deoiled cake through compost production. The deoiled cake was composted with three different components inorder to determine the role of compost on plant growth regulation in *Brassica compestris* under water deficit conditions. NRA, proline, photosynthetic pigments and SOD was assessed under irrigated and water deficit conditions. During stress period, increase in photosynthetic pigments and NR activity was noticed, in composts treated samples over control, on the contrary, significant alleviation in proline and SOD production in control plants under water deficit condition over irrigated as well as compost amended stressed plants. This study provides evidence for a beneficial effect of compost in enhancing drought tolerance under water deficit conditions.

Keywords: *Brassica Juncea*, compost, drought, *Jatropha curcas*.

Introduction

Brassica juncea is a widely distributed and economically important oil seed crop, grown all over the country. The expected demand for oilseeds in India by 2020 will be around 34 million tonnes, out of which about 41% is to be met by mustard seeds. Drought is the most serious natural disaster greatly affecting crop production in India. It is frequently occurred as a natural disaster and in many circumstances affects a large area under agriculture in India. The crops grown in such areas often face situations of short to long periods of drought and therefore, it is considered as one of the major constraints for the productivity of crops. Nevertheless, yield of oilseeds have been fluctuating because of water deficit[1]. Compost can be considered as a soil conditioner that contributes to soil fertility, structure, porosity, organic matter, water holding capacity and disease suppression[2]. Thus, compost has the potential to decrease abiotic stress of cultivated plants.

Under a survey conducted by Ministry of Rural Development and National Remote Sensing Centre in 2010[3], it was noticed that, India has around 316.65 million hectares, geographical area and out of which 63.85 million hectares (20.16% of the total geographical area) is only wasteland. Therefore, the National strategies on Bio-fuels promotes the use of wasteland for growing non-edible oilseeds and about 20% of this policy can be achieved by 2017[4]. Jatropha has gained widespread attention in India as this non-food biodiesel crop, was considered as one of the best candidates in many developing countries for future biodiesel production[5]. Extraction of oil from jatropha seeds generate large amount of jatropha deoiled cake waste, which contain about 70 - 80 percent of the total mass of the seeds, depending on the extraction rate[6]. However, due to its toxic nature it is unsuitable for animal feed and therefore, in future, disposal of jatropha deoiled cake will create problem. At the same time, it is a rich source of nitrogen, phosphorous and potassium, which makes it valuable organic manure and shows its potential as a fertilizer[7,8]. Finding a lower cost, environmentally sustainable, long-term solution for handling jatropha deoiled cake is therefore of critical importance. Keeping in view, this study was undertaken and two different jatropha deoiled cake based composts were prepared. This nutrient-enriched compost may be used further as manure for organic farming and should be amended in soil through field trials followed by validation prior to recommendation[9]. The aim of this work was to evaluate the effects of Jatropha (*Jatropha curcas* L.) de-oiled cake based composts for sustaining growth of *Brassica* under drought conditions on the activity of antioxidant enzymes (superoxide dismutase), proline content, nitrate reductase

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activity and chlorophyll content in *Brassica Juncea* varieties in order to better understand their differences in water stress condition.

Materials and Methods

Jatropha deoiled cake based composts:

Two different composts were used: (i) mixture of jatropha (*Jatropha curcas* L.) de-oiled cake, rice straw and cow dung (JRsC,) supplied by The Energy and Resource Institute (TERI), Delhi, India (ii) mixture of jatropha (*Jatropha curcas* L.) 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[10]. 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 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 airdried.

Plant growth and treatments:

The seeds of Brassica Juncea were sown in plastic pots filled with soil and compost mixture (5:1) and experiment was carried out 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 27°C and 60 % relative humidity. The work was conducted in factorial and completely randomized design with four replications of each treatment (with compost) and control (without compost). Water holding capacity of soil was determined before adding compost. The pots (treatment and control) were irrigated regularly with equal amount of water on the basis of determined water holding capacity. Irrigation was interrupted to induce drought stress at 30 days old plants in half of the pots only, whereas, another half pots were regularly irrigated as per water holding capacity. First harvest was made after 4 days of water stress. Second and third harvesting was made after 8 and 10 days, respectively using same protocol.

Measurement of Photosynthetic Pigments:

Five hundred mg of fresh leaf were collected from the second and third nodes of the shoot tip. Extraction of pigments was achieved with 80% acetone in ice bucket. Extract was centrifuged at 5000 rpm for 10 minutes. Extraction was done twice. 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)[11].

Nitrate reductase activity:

Five hundred mg fresh leaves were taken in black vials of 20 ml, containing 8.0 ml 0.1 M sodium phosphate buffer (pH 7.4), 1.0 ml 0.2 M KNO_3 and 1.0ml 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 of 1% sulphanilamide in 1 N HCl and 1.0 ml 0f 0.2 % N-(1-naphthyl) ethylenediamine-dihydrochloride (NED) was added in 1.0 ml of aliquot from the incubation mixture. After 20 minutes, absorbance was read by a spectrophotometer (Ray Leigh, UV-2601) at 540 nm and was calculated as millimole NO_2^- hr^{-1} g^{-1} FW in vivo following the method of Srivastava (1975)[12]. The colour achieved was due to formation of diazo compound with sulphanilamide and nitrite, which is coupled with NED to give a red colour.

Proline content:

Proline content was determined following the method of Bates et al, (1973)[13]. The young expanded leaves (200mg) was homogenized in 4 ml sulfosalicylic acid (3%) and centrifuged at 10,000g for 30 min. Two ml extract supernatant was added with 2 ml glacial acetic acid and 2 ml ninhydrin reagent. 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 at 520 nm using toluene as a blank. Proline content (µmol g^{-1} fr. wt.) was quantified using the ninhydrin acid reagent method by using L-proline as a standard.

Superoxide Dismutase:

Total SOD activity was determined by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium chloride (NBT), as described by Giannopolitis and Ries (1977)[14]. 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 the 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 the NBT photo reduction rate and the results expressed as U mg^{-1} of fresh weight.

Data analysis:

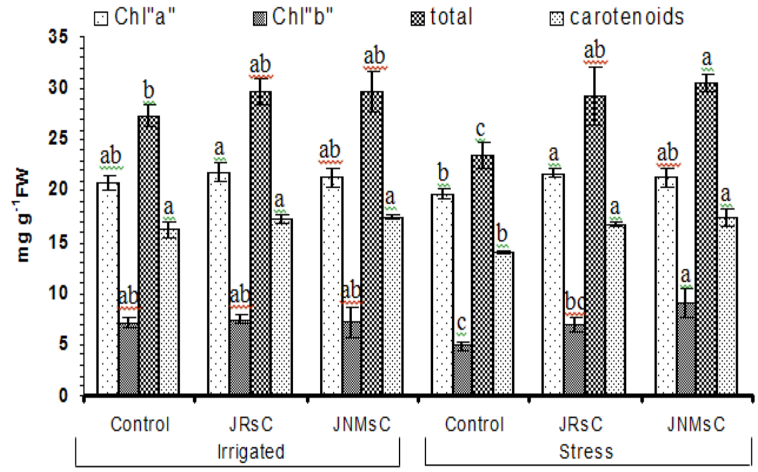
The statistical evaluation of complete data was done and all analyses were performed based on four replicate pots. Data were subjected to one-way analysis of variance (ANOVA) with different physiological and biochemical parameters (JMP 5.0, SAS Institute, Cary, NC, USA) to analyze the effect of each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

Results and Discussion

Photosynthetic pigments:

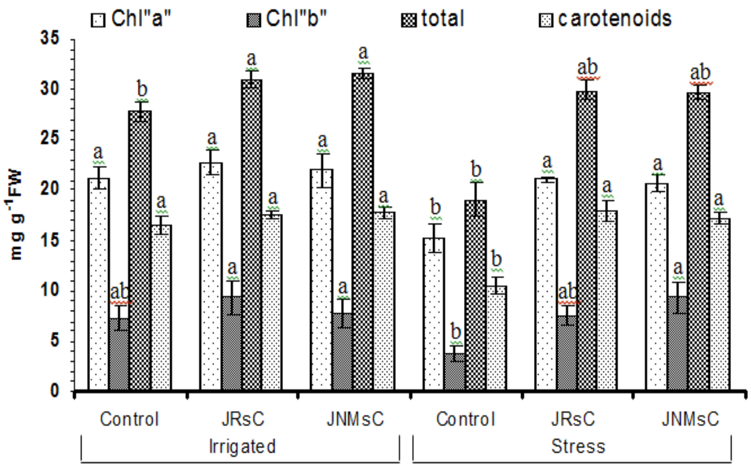
Result depicts significantly higher values in compost treated *Brassica compestris* over to control after 4, 8 and 10 days of interrupting irrigation. However, Chl 'b' and Chl 't' was found significantly higher in compost amended samples in comparison to control after 4 days of stress period. Whereas, after 8 and 10 days of stress, Chl 'a', Chl 'b' and Chl 't' shows significant decline in control sample in comparison to JRsC and JNMsC samples (Fig: 1). Furthermore, carotenoids content declined non-significantly in control over to compost amended *Brassica compestris* under stress period of 4, 8 and 10 days.

Fig.1: Effects of composts on photosynthetic pigment composition in *Brassica campestris* under drought stress of 4 days



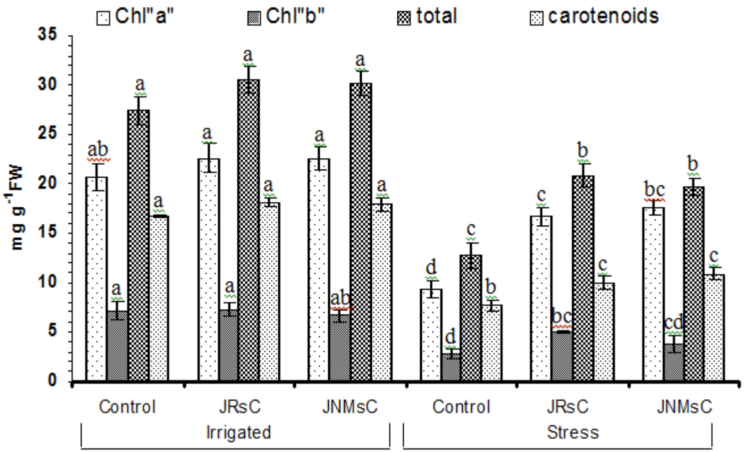
*Chlorophyll a (Chl "a"), Chlorophyll b (Chl "b"), Total Chlorophyll (total), Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)
*Level not connected by same letter are significantly different

Fig.2: Effects of composts on photosynthetic pigment composition in *Brassica campestris* under drought stress of 8 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 *Brassica campestris* under drought stress of 10 days



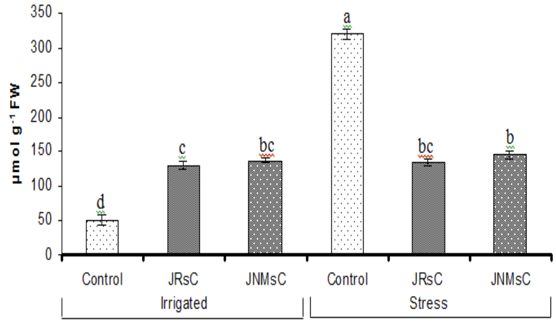
*Chlorophyll a (Chl "a"), Chlorophyll b (Chl "b"), Total Chlorophyll (total), Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)
*Level not connected by same letter are significantly different

This variation in chlorophyll level during stress depends on the severity and duration of water deficit[15,16]. It was documented that under stress stomata get blocked, which leads to a decrease in absorbing carbon dioxide, while on the other hand the plants take lot of energy to absorb water, which may cause depletion in producing photosynthetic matter. At the same time, results also define maximum fluctuation in chlorophyll 'b' under stress conditions (Fig: 1-3). The present finding is in agreement with the finding of Netando *et al.* (2004)[17] and the possible reason for this may be due to the structural variation and localization of chlorophyll 'b' in antennae complex[18]. Decline in photosynthetic components in control under stress condition could be due to disruption in the components of plant's photosynthetic system, such as membrane integrity, which, probably decrease the photosynthetic capacity of plant[19]. However, the higher total chlorophyll concentration in plant leaves was observed in composts (JRsC and JNMsC) treatment compared to control as they had a potential for improving plant growth. Jatropha deolied based compost helps in soil restoration and contributes in management of structure, porosity, and water holding capacity. It also plays a vital role 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 better photosynthetic rate.

Proline content:

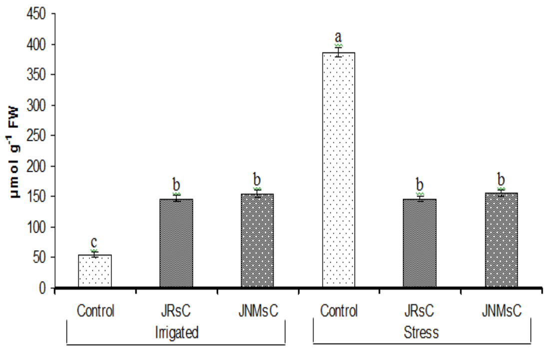
After 4, 8 and 10 days of irrigation, proline content increased significantly in control compared to the plants supplied with JRsC and JNMsC. However, the same results were obtained under stress condition. Furthermore, proline content increased significantly under stress conditions over irrigated in all three treatments i.e. control, JNMsC and JRsC (Fig: 4 and 5). However, in 10 days harvested samples, non-significant results were observed between JRsC and JNMsC under stress condition (Fig: 6).

Fig.4: Effects of composts on proline content in *Brassica campestris* under drought stress of 4 days.



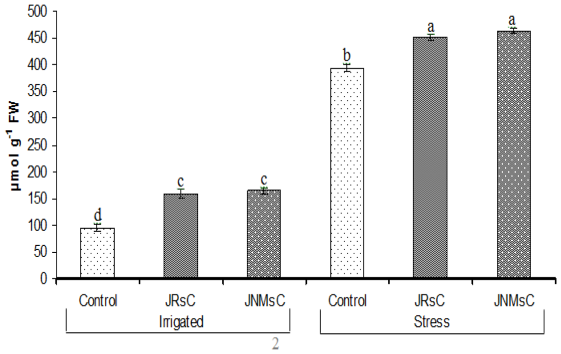
*Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)
*Level not connected by same letter are significantly different

Fig.5: Effects of composts on proline content in *Brassica campestris* under drought stress of 8 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 *Brassica campestris* under drought stress of 10 days



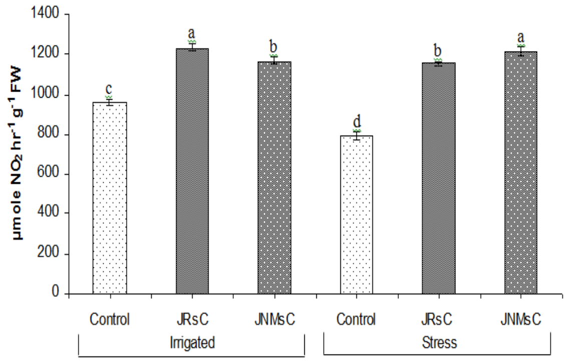
*Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)
*Level not connected by same letter are significantly different

Proline has been selected to evaluate the abiotic stress studies as it increases comparatively faster than other amino acids under stressed conditions. 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 i.e. JRsC and JNMsC under stressed condition may be due to low injury in compost treated plants as a result of drought avoidance. A possible reason for the increased levels of proline during water stress could be an alteration in the activities of the enzymes involved in the biosynthesis and degradation of proline[20]. This mechanism results in the higher accumulation of low molecular weight water soluble, nitrogen containing metabolites, like proline in their cells for osmotic adjustment[21]. However, present results were in agreement with the study carried out by Lutts and his coworker in 1996[22]. They also concluded in their reported that abiotic tolerant cultivated rice accumulates less free proline than stress sensitive one.

Nitrate reductase activity:

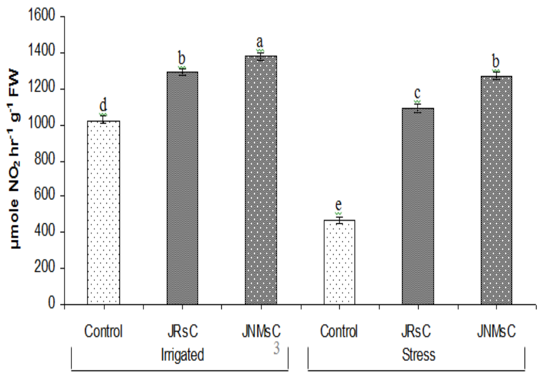
Over all NR activity in irrigated samples was found significantly increased JRsC and JNMsC over to control in all 4, 8 and 10 days harvested samples of Brassica. On the contrary, significant decrease was observed in control over to JRsC and JNMsC amended samples under stress in all undertaken time intervals. Furthermore, it was concluded that, JNMsC amended samples shows significantly higher values over JRsC in most of the case (Fig: 7-9).

Fig.7: Effects of composts on nitrate reductase in *Brassica campestris* under drought stress of 4 days



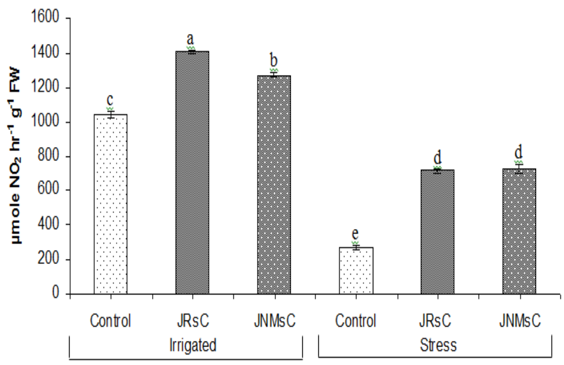
*Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)
*Level not connected by same letter are significantly different

Fig.8: Effects of composts on nitrate reductase in *Brassica campestris* under drought stress of 8 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 *Brassica campestris* under drought stress of 10 days.



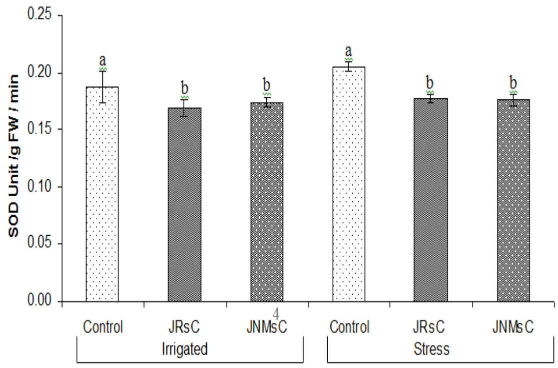
*Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)
*Level not connected by same letter are significantly different

Nitrate reductase activity is assumed to be the rate-limiting step of the nitrateassimilating pathway and plays a central role in plant primary metabolism and exhibits complex regulation mechanism for its catalytic activity[23,24]. The process of N assimilation starts with the reduction of NO_3 to NO_2 by nitrate reductase, this enzyme serves as a rate limiting factor in the N assimilation process and drastically slowed down by drought stress[25]. However, in present study, NR activity gets enhanced in compost over to control in irrigated samples which means that, compost improved nutritional status and nitrogen assimilation rate of water stressed plants.

Superoxide dismutase:

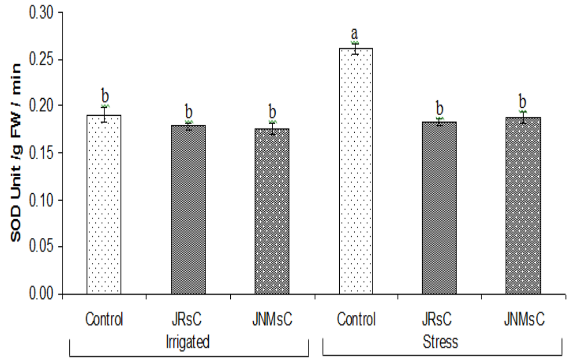
SOD activity increased significantly in control under stress condition in comparison to control of irrigation, whereas, non-significant difference was observed between control and JNMsC amended cultivars under stress (Fig: 10-12). However, 8 and 10 days of harvested samples depict significant increase in control over compost amended cultivars under stress. However, in 8 and 10 days harvested samples, non-significant results were observed between JRsC and JNMsC under stress condition (Fig: 11 and 12).

Fig.10. Effects of composts on superoxide dismutase in *Brassica campestris* under drought stress of 4 days



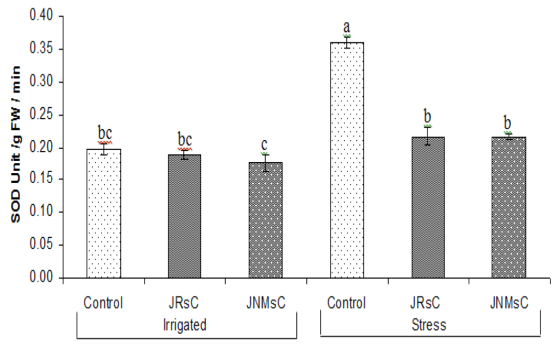
*Jatropha deoiled cake, Rice straw and Cow dung (JRsC) and Jatropha deoiled cake, Neem cake, Mushroom spent and *Cuscuta reflexa* (JNMsC)
*Level not connected by same letter is significantly different

Fig.11: Effects of composts on superoxide dismutase in *Brassica campestris* under drought stress of 8 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 is significantly different

Fig.12: Effects of composts on superoxide dismutase in *Brassica campestris* 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

Results depicted maximum SOD activity in control of stress over compost containing plants, which means that SOD activity increases in plants during stress period and plays an important role as the protective agent. This may be due to ROS scavenging mechanism of plant which acts as defense enzymes and protect plant from oxygen toxicity caused by the abiotic stress[26]. High concentration of ROS disrupts the normal physiological and cellular functions; therefore, detoxification of excess ROS is very essential[27]. Golkar and his coworkers in 2009[28] studied and defined that, greater SOD is associated with decreased damage to photosynthetic apparatus.

Conclusion

In present scenario drought is an important environmental constraint that limits plant growth and crop production. 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 etc. Moderate to severe water stress drastically affects various morpho-physiological traits in crops such as chlorophyll content, amino acids, nitrogen and many other productive parameters. 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. Compost plays a vital role in avoidance and tolerance of drought stress as it provides plant nutrients, organic matter etc., and have the potential to improve the drought tolerance of crops. Hence, its use is quite feasible and a viable method in recycling both agricultural and industrial residues in large quantities. The data presented here reflect the response and importance of compost on plants under water deficit stress. Further investigations are required to ascertain this conclusion for betterment of agricultural crops even under adverse environmental conditions.

Acknowledgements

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