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Research Article

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Effect of Foliar Nutrition on Antioxident Enzymes, Photosynthetic Rate, Dry Matter Production and Yield of Mung Bean under Receding Soil Moisture Condition

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ABSTRACT

A field experiment was conducted during Rabi season of 2012-13 at Regional Agricultural Research Station, Lam, Guntur in field No.3, with an aim to find out effect of foliar nutrition on antioxident enzymes, photosynthetic rate, dry matter production and yield of mung bean under receding soil moisture condition in split plot design with irrigation and no irrigation as main treatments and foliar sprays as sub treatments. Under receding soil moisture condition (moisture stress) KNO_3 @ 1% proved superior over other foliar sprays by recording more plant height, leaf area, shoot dry weight, and photosynthetic rate by maintaining high leaf proline content, peroxidase activity, SOD activity. Under irrigated conditions urea @2% recorded higher yield. KNO_3 @ 1% gave higher yields under receding soil moisture condition compared to other foliar sprays. Among all treatments controlled (no spray) under unirrigated conditions recorded lower yields due to moisture stress and nutrient deficiency.

Keywords*: Receding soil moisture, Foliar spray, Antioxident, KNO_3, Mung bean.*

INTRODUCTION

Pulses provide rich and cheap source of protein, particularly to the vegetarians and the poor, who constitute the bulk population in India. They contain 30 % of proteins, which are nearly three times as much as cereals.

Blackgram (*Vigna mungo* (L.) Hepper) is the fourth important pulse crop in India and second most important in Andhra Pradesh in terms of extent of cultivation. Seasonal variability in available moisture is the major constraint to production under rained farming. The erratic and low rainfall along with high temperature in the rainfed farming induces periods of water stress during crop growth. Thus the ability of the crop to grow and yield in such environments depends upon the relative performance of cultivars under drought. In A.P state blackgram is grown during *rabi* under receding soil moisture conditions without any irrigation. As a result there was water deficit for the crop at critical stages, which affects the nutrient uptake, ultimately causing yield reduction. To increase the yield during drought conditions we have to take into consideration not only the normalization of plant water regime, but also the normalization of plant feeding and elimination of created deficiencies of some elements. A suitable way of plant feeding during and after drought is through foliar nutrition. Keeping this in view an investigation was carried to know the response of blackgram to foliar nutrition under receding soil moisture condition.

MATERIAL AND METHODS

Blackgram seeds of PU 31 variety were sown in blackcotton soils on October 2012 at RARS, LAM, Guntur. The average temperature during the crop period varied from 31.7 ^{o}C and 18.9 ^{o}C. The total amount of rainfall received during the crop duration was 215.7mm.

Average relative humidity was 94.2% to 57.2%. Sowing was done with a spacing of 30 cm x 10 cm in 3m x4m ($12m^2$) plot. The experiment was arranged as split plot design with three replications keeping irrigation (M_1) and unirrigation (M_2) as main plots and foliar spray of KNO_3 @ 1%, Urea @ 2%, DAP @ 2%, K_2SO_4 @ 1%, Triacontanol @ 1 ppm, water spray and control (no spray) as subplots. Nitrogen and phosphorus fertilizers were applied as per the recommendation (20 kg N and 50 kg P_2O_5 ha^{-1}) before sowing of the crop. Experimental plots were protected from pest and diseases by spraying of Monocrotophos @ 2 ml l^{-1} at the initial stage of the crop growth. Manual weeding was done at 15 days interval up to pod setting. Supplemental irrigation for irrigated main plot was given at 33 DAS and unirrigated main plot was maintained without any irrigation. Soil moisture percent was measured in both irrigated and unirrigated main treatments. Foliar spray was done at flowering and pod initiation stages. Leaf area and shoot dry weight ware measured by destructive growth analysis. Total leaf area per plant (cm^2) was measured by using LI-COR LI-3100C leaf area meter. Water potential of leaves was measured by using WESCOR's water potential system (PSΨPRO). Photosynthetic rate of leaves was measured by using LI-COR LI-6400XT portable photosynthetic system. Canopy temperature was measured by using Raytek infrared thermometer. Soil moisture was measured at 10, 20 30 and 60 cm depth using profile probe type PR2 and soil moisture meter type HM2 of Delta-T devices at weekly intervals from 25 DAS to 61 DAS.

Proline content (µg g^{-1} fresh weight): Proline content in the leaves was determined after imposing treatments in both irrigated and unirrigated conditions by following the method of Bates *et al.* (1972). Fresh leaf material, weighing 0.5 g was homogenized in 10 ml of 3 % aqueous sulphosalicylic acid and the homogenate was filtered through Whatman No.2 filter paper. An aliquot of 2 ml of the filtrate was reacted with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 h at 100°C and the reaction was terminated by keeping in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed vigorously with a test tube stirrer for 15 sec. The chromophore containing toluene was separated in a separating funnel. The separated aqueous phase was warmed to room temperature and the absorbance was read at 530 nm using toluene as a blank. Proline concentration was determined from a standard curve of proline and was expressed as µg g^{-1}. Fresh proline (AR) was used for the preparation of standard curve.

$$\text{Proline (µg g}^{-1}\text{ fresh weight)} = \frac{\text{OD x 36.231 x V}}{\text{Y x W}}$$

(OD = Optical density at 520 nm ,V = Final volume of extract, Y = Volume of aliquot taken, W = Fresh weight of the plant material.)

Peroxidase activity: Peroxidase activity in the leaves was estimated after imposing treatments in both irrigated and unirrigated conditions by following the method of Malik and Singh (1994). Peroxidase activity was estimated by the degradation of H_2O_2 substrate. Fresh leaf sample of 0.2 g was grounded in 2 ml of phosphate buffer of pH 7.8 in chilled pestle and motor. The sample was collected into centrifuge tubes and centrifuged at 4^0 C at 1500 rpm for 10 minutes. Supernatant was used as enzyme extract. Enzyme units were expressed as units per ml of extract per minute (number of units of substrate degraded by the enzyme per minute)

$$\text{Enzyme activity} = \frac{V_t}{V_S \times \varepsilon_0 \times \Delta T} \times \text{Dilution factor}$$

(V_t = Total volume, V_S = Volume of sample, ΔT = Time difference, $\varepsilon_0 = 6.39$)

Superoxide Dismutase (SOD) activity: SOD activity in the leaves was estimated after foliar spray in both irrigated and unirrigated conditions by following the method of Bayer and Fridovich (1987). SOD activity was measured by monitoring the inhibition of nitro blue tetrazolioum (NBT) reduction at 560 nm.

Ground 0.2 g of leaf tissue in chilled mortar and pistle in extraction buffer (100 ml of 50 mM potassium phosphate buffer pH -7.5), 1 mM EDTA and 1% (w/v) PVP). The homogenate is centrifuged at 15,000 rpm for 20 minutes at 4 ^{0}C and the supernatant is used for enzyme assays. Under the experimental condition, the initial rate of reaction, as measured by the difference in increase of absorbance at 560 nm in the presence and absence of extract, was proportional to the amount of enzyme. The unit of SOD activity was defined as the amount of enzyme that inhibits the NBT photo reduction by 50%. SOD activity values are given in units per ml of extract per minute.

Yield and its components such as number of pods per plant and test weight (100 seed weight) were measured at harvesting stage. The experimental data were analysed statistically by the method of analysis of variance procedure as suggested by Panse and sukhatme (1978). Statistical significance was tested by 'F' value at 5 % level of probability. Critical difference at 5 % was worked out.

Fig.1. Soil moisture (% volume) under irrigated condition

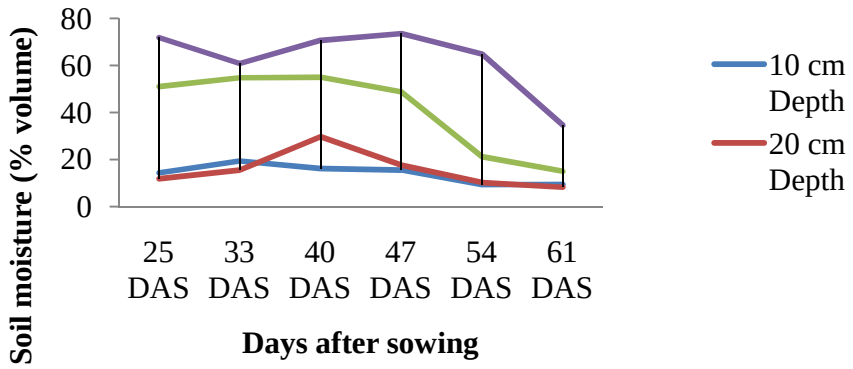
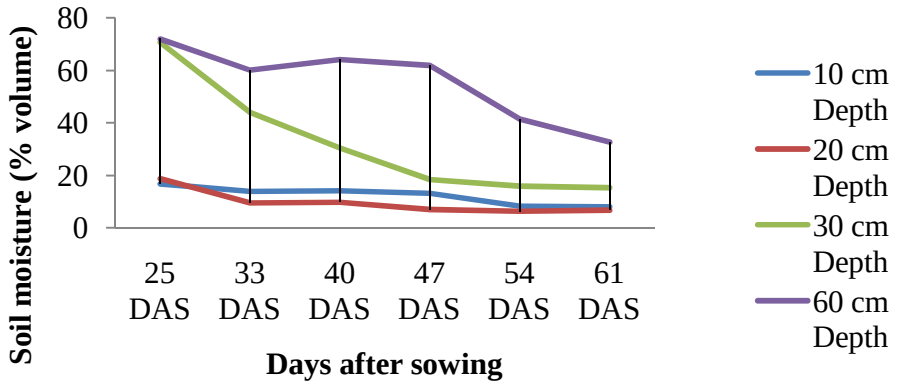


Fig. 2. Soil moisture (% volume) under unirrigated condition



RESULTS AND DISCUSSIONS

Plant height (cm), Leaf area(cm^2 plant^{-1}), Shoot dry weight (g pant^{-1})

The data on the influence of application of foliar chemicals on plant height, leaf area and shoot dry weight of blackgram are presented in table number one. All these three parameters shows a significant difference among main plots and subplots.

Plant height after foliar spray under irrigated condition was 19.55 cm and under unirrigated condition it was 17.43 cm. Reduction in plant height due to drought after foliar spray was 10.84%. Reduction in plant height was due to diversion of assimilates from stem and utilised them for increased root growth in order increase the water absorption. The results of reduction in plant height due to drought ware in conformity with Ali *et al.*[3] in maize and Bardhan *et al.*[5] in chickpea. Among interactions under irrigated conditions foliar spray of urea @ 2% recorded highest plant height (21.29 cm) and under unirrigated condition, foliar spray of KNO_3 @ 1% recorded significantly higher plant height (19.45 cm) which is on par with K_2SO_4 @ 1% and urea spray @ 2% . Increase in plant height was due to availability of Nitrogen and potassium to plants through foliar spray. Potassium regulates the osmotic turgor of cells and water balance which is driving force for cell division and elongation[5]. Similar results of increase in plant height due to foliar nutrition of KNO_3 and potassium solution during drought was revealed by Bardhan *et al.*[5] in chickpea and Besma *et al.*[7] in potato respectively.

Leaf area after foliar spray under irrigated condition was 441.03 cm^2 per plant and under unirrigated condition leaf area was 383.50 cm^2 per plant. The decrease in leaf area due to drought was 13.04 % after foliar spray because of drought stress. The results of reduced leaf area in water stressed plants was due to accelerated senescence and low turgor potentials which is driving force for cell division and cell elongation. Similar results of decrease in leaf area due to drought was revealed by Ali *et al.*[3] in maize and Maiti *et al.*[15] in various crops. Among interactions under irrigated condition urea spray @ 2% recorded highest leaf area (520.83 cm^2 $plant^{-1}$) and in unirrigated condition KNO_3 @ 1% recorded highest leaf area (412.5 cm^2 $plant^{-1}$). Lower leaf area was recorded by control (338.83 cm^2 $plant^{-1}$). Potassium is essential to obtain maximum leaf extension and stem elongation. Potassium regulates the osmotic turgor of cells and water balance which is driving force for cell division and elongation. A similar result of increase in leaf area due to foliar spray of potassium was reported by Besma *et al.*[7] in potato.

Regarding shoot dry weight under irrigated condition it was 3.86 g per plant and under unirrigated condition shoot dry weight was 3.39 g per plant. The decrease in shoot dry weight due to drought, after foliar spray was 12.18 %. Reduction in shoot dry weight under drought was due to reduced shoot growth, reduced leaf area, number of leaves, plant height and increased senescence. Similar results of decrease in shoot dry weight due to drought was revealed by Ali *et al.*[3] in maize and Abdullahil *et al.*[1] in wheat. Among interactions irrigated condition urea spray @ 2% recorded significantly higher shoot dry weight (4.83 g $plant^{-1}$) and under unirrigated condition KNO_3 @ 1% recorded higher shoot dry weight (3.77 g $plant^{-1}$) which is on par with K_2SO_4 @ 1% . Lower shoot dry weight was recorded by control (2.84 g $plant^{-1}$). KNO_3 marginally delayed the flowering. Delay in flowering would facilitate whole dry matter production. So foliar application of KNO_3 contribute in dry matter production (up to some extent) as indicated by delayed flowering[5]. Similar results of increase in shoot dry weight due to foliar spray of potassium under drought conditions was reported by Abdullahil *et al.*[1] in wheat and Bardhan *et al.*[5] in chickpea.

Canopy temperature (^{0}C)

Canopy temperature showed significant increase under unirrigated condition when compared with irrigated condition. After foliar spray under irrigated condition canopy temperature was 29.05 ^{0}C and under unirrigated condition it was 31.38 ^{0}C. The increase in canopy temperature due to drought, after foliar spray was 8.0%.

Increase in leaf and canopy temperatures under drought was due to inhibited transpiration and increase in the boundary layer resistance to transpiration in maize[10]. Leaf and canopy temperatures increased due to increased respiration and decreased transpiration resulted from stomatal closure in wheat[25]. Similar results of increase in canopy temperature due to drought was reported by Moradi *et al*.[18] in mungbean, Ali *et al*.[3] in maize and Talebi[28] in wheat.

Among interaction there was no significant difference in canopy temperature with foliar spray. Under irrigated and unirrigated condition higher canopy temperature was recorded in control.

Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})

Photosynthetic rate under unirrigated conditions decreased significantly when compared with irrigated conditions. Under unirrigated condition photosynthetic rate was 36.02 µmol CO_2 m^{-2} s^{-1} and under irrigated condition it was 39.94 µmol CO_2 m^{-2} s^{-1}. The decrease in photosynthetic rate was 9.81 %. Reduction in photosynthetic rate by drought stress is due to stomatal (stomatal closure) and nonstomatal (impairments of metabolic processes) factors[14]. Photosynthesis can be inhibited even when the stomatal influence is eliminated (leaf discs without epidermis), suggesting that factors other than low CO_2 availability affect photosynthesis under drought conditions[27]. Similar results of decrease in photosynthetic rate due to drought was reported in mungbean[18], in tobacco[22] and in chickpea[13].

Under unirrigated conditions all treatments showed significant increase in photosynthetic rate when compared with control. KNO_3 @ 1% spray recorded maximum photosynthetic rate (39.63 µmol CO_2 m^{-2} s^{-1}) and lower photosynthetic rate was recorded by control (29.48 µmol CO_2 m^{-2} s^{-1}). The reason for the enhanced need for K by plants suffering from environmental stress like drought appears to be related to the fact that K is required for maintenance of photosynthetic CO_2 fixation. Drought stress is associated with stomatal closure and thereby with decreased CO_2 fixation. Formation of ROS is intensified because of inhibited CO_2 reduction by drought stress. Obviously, formation of ROS under drought stress would be dramatic in plants exposed to high light intensity, with concomitant severe oxidative damage to chloroplasts. Increase in ROS production in drought-stressed plants is well known and related to impairment in photosynthesis and associated disturbances in carbohydrate metabolism[9]. Under irrigated conditions all treatments showed significant increase in photosynthetic rate when compared with control. Urea @ 1% spray recorded maximum photosynthetic rate (43.94 µmol CO_2 m^{-2} s^{-1}).

Proline content (µg g^{-1} FW)

Total proline content showed a significant increase under unirrigated condition when compared with irrigated condition. After foliar spray under irrigated condition total proline content was 2.96 µg g^{-1} FW and under unirrigated condition it was 5.16 µg g^{-1} FW. The increase in total proline content due to drought after foliar spray was 74.32 %. Accumulation of proline in plants under stress is a result of the reciprocal regulation of two pathways i.e., increased expression of proline synthesis enzymes and repressed activity of proline degradation. This leads to a "proline cycle", the homeostasis of which depends on the physiological state of tissue[17] in maize. Proline accumulation is a mechanism for plants adaptation to abiotic stress conditions. Other roles for proline have been proposed, including stabilization of macromolecules, a sink for excess reductant and a store of carbon and nitrogen for use after relief of water deficit in mungbean[29]. Similar results of increase in proline content during drought was reported by Mafakheri *et al*.[14] in chickpea and Maiti *et al*.[15] in various crops. Under unirrigated condition KNO_3 @ 1% recorded significantly higher total proline content (5.64 µg g^{-1} FW).

Foliar spray of potassium induces proline synthesis during drought and this accumulation of proline might have served as a compatible solute[7]. Similar results of increase in proline content during drought due to foliar spray of KNO_3 was reported by Thalooth *et al.*[29] in mungbean. Under irrigated condition foliar spray of KNO_3 @ 1% recorded significantly higher total proline content (3.25 µg g^{-1} FW) which is on par with K_2SO_4 @ 1% (3.20 µg g^{-1} FW).

Peroxidase and SOD activity (units per ml of extract)

Regarding peroxidase and SOD activity there was a significant difference among the interactions between irrigated and unirrigated foliar treatments. Total peroxidase and SOD activity showed a significant increase under unirrigated condition when compared with irrigated condition. After foliar spray under irrigated condition total peroxidase activity was 338.09 units per ml of extract and under unirrigated condition it was 362.24 units per ml of extract. The increase in total peroxidase activity due to drought after foliar spray was 7.14 %. Under irrigated condition SOD activity was 25.57 units per ml of extract and under unirrigated condition it was 37.99 units per ml of extract. The increase in SOD activity due to drought after foliar spray was 48.57 %. Drought results in the increased generation of reactive oxygen species (ROS) due to energy accumulation in stressed plants.

Plants have developed a wide range of adaptive/resistance mechanisms to maintain productivity and ensure survival under drought stress condition. To reduce the toxicity of ROS, plant cells have developed an antioxidative system, consisting of low molecular- weight antioxidants and as well as protective enzymes like, peroxidase and superoxide dismutase. Similar results of increase in peroxidase and SOD activity during drought was reported by Salekjalali *et al.*,[23] in barley, Abedi and Hassan[2] in rape seed and Mousa *et al.*,[19] in maize. Among interactions under unirrigated condition foliar spray of KNO_3 @ 1% recorded significantly higher peroxidase (382.0 units per ml of extract) and SOD (42.0 units per ml of extract) activity. Lower peroxidase and SOD activity was observed in water spray. Application of macro-nutrients like N and K during drought reduced the toxicity of ROS by increasing the concentration of antioxidants like peroxidase and SOD in the plant cells[30]. Among interactions, under irrigated condition foliar spray of KNO_3 @ 1% recorded significantly higher peroxidase and SOD activity.

Seed yield (kg ha^{-1})

Regarding seed yield there was a significant difference among the interactions. Seed yield showed a significant decrease under unirrigated condition when compared with irrigated condition. Under irrigated condition seed yield was 707.07 kg ha^{-1} and under unirrigated condition it was 548.43 kg ha^{-1}. The decrease in seed yield due to drought was 22.44 %. Similar results of decrease in seed yield due to drought were reported by Talooth *et al.*[29] in mungbean and Mafakheri *et al.*[14] in chickpea. Among interactions, under unirrigated condition KNO_3 @ 1% recorded significantly higher seed yield (604.02 kg ha^{-1}). Lower seed yield was observed in control (490.93 kg ha^{-1}). The increase in yield due to KNO_3 @ 1% spray under drought was 23.04 % when compared with control. Similar results of increase in seed yield due to foliar spray of KNO_3 or potassium spray under drought was reported by Jayaramireddy *et al.*[11] in mungbean, Bardhan *et al.*[5] in chickpea and Abdullahil *et al.*[1] in wheat. Under irrigated condition foliar spray of urea @ 2% recorded significantly higher seed yield (792.17 kg ha^{-1}). Similar results of increase in seed yield due to foliar spray of urea under normal irrigated condition was reported by Rajavel *et al.*[21] in mungbean, Sritharan *et al.*[26] in mungbean and Bahr[4] in chickpea.

Table 1: Effect of foliar nutrition on plant height, leaf area and shoot dry weight under receding soil moisture conditions

Treatments	Plant height (Cm)			Leaf area (cm^2 per plant)			Shoot dry weight (g per plant)		
	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean
KNO_3 @ 1%	20.97	19.45	20.21	486.10	412.50	449.30	4.13	3.77	3.95
Urea @ 2%	21.29	18.80	20.04	520.83	408.07	464.45	4.83	3.61	4.22
DAP@ 2%	19.70	17.44	18.57	473.19	387.57	430.38	4.00	3.40	3.70
K_2SO_4 @ 1%	20.46	19.14	19.80	437.60	390.93	414.27	4.07	3.65	3.86
Tricantanol @ 1 ppm	20.00	18.16	19.08	444.95	391.60	418.28	3.70	3.38	3.54
Water	17.87	15.27	16.57	378.53	354.97	366.75	3.35	3.10	3.23
No spray	16.54	13.71	15.13	345.97	338.83	342.40	2.93	2.84	2.89
Mean	19.55	17.43		441.03	383.50		3.86	3.39	
	SEM ±	CD	CV%	SEM ±	CD	CV%	SEM ±	CD	CV%
Main plot	0.08	0.49	8.62	0.84	5.10	18.91	0.07	0.41	16.22
Sub plot	0.27	0.80	15.57	1.27	3.70	15.27	0.63	0.18	8.05
Interaction M x S	0.39	1.13		1.79	5.22		0.09	0.26	

M_1 = Irrigation M_2 = Unirrigation S x M = Sub plot means at fixed level of main plots

Table 2: Effect of foliar nutrition on proline content, peroxidase activity and SOD activity under receding soil moisture conditions

Treatments	Proline content (µg/g FW)			Peroxidase activity (units/ml extract)			SOD activity (units/ml extract)		
	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean
KNO_3 @ 1%	3.25	5.64	4.45	357.70	382.03	369.87	28.47	42.00	35.23
Urea @ 2%	2.90	4.51	3.71	346.43	362.50	354.47	25.73	38.20	31.97
DAP@ 2%	3.03	5.08	4.05	335.47	358.47	346.97	25.33	38.33	31.83
K_2SO_4 @ 1%	3.20	5.35	4.28	350.03	375.57	362.80	26.23	41.80	34.02
Tricantanol @ 1 ppm	2.45	5.26	3.86	327.07	352.93	340.00	25.27	37.30	31.28
Water	2.84	5.00	3.92	324.90	349.97	337.43	24.23	34.87	29.55
No spray	3.02	5.26	4.14	325.00	354.23	339.62	23.70	33.43	28.57
Mean	2.955	5.158		338.09	362.24		25.57	37.99	
	SEM ±	CD	CV%	SEM ±	CD	CV%	SEM ±	CD	CV%
Main plot	0.01	0.07	2.53	0.17	1.03	4.16	0.04	0.26	3.43
Sub plot	0.04	0.11	4.45	0.42	1.23	5.53	0.16	0.48	7.17
Interaction S x M	0.05	0.15		0.59	1.74		0.23	0.68	

M_1 = Irrigation M_2 = Unirrigation S x M = Sub plot means at fixed level of main plots

Table 3: Effect of foliar nutrition on water potential, Photosynthetic rate and Protein content under receding soil moisture conditions

Treatments	canopy temperature (^{0}C)			Photosynthetic rate (µmol m^{-2} s^{-1} CO_2)			Grain yield (kg/ha)		
	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean
KNO_3 @ 1%	27.90	29.43	28.67	41.96	39.63	40.80	770.27	604.02	687.15
Urea @ 2%	28.70	31.43	30.07	43.94	38.24	41.09	792.17	585.30	688.73
DAP@ 2%	29.30	32.27	30.78	42.31	35.41	38.86	755.92	569.91	662.92
K_2SO_4 @ 1%	28.17	30.47	29.32	41.28	39.22	40.25	714.77	577.82	646.29
Tricantanol @ 1 ppm	29.00	30.70	29.85	40.88	35.75	38.32	663.87	518.27	591.07
Water	29.40	32.23	30.82	35.76	34.43	35.10	643.50	492.77	568.13
No spray	30.90	33.10	32.00	33.45	29.48	31.47	609.00	490.93	549.97
Mean	29.05	31.38		39.94	36.02		707.07	548.43	
	SEM ±	CD @ 5%	CV%	SEM ±	CD	CV%	SEM ±	CD	CV%
Main plot	0.17	1.0	13.72	0.14	0.84	10.27	1.22	7.45	22.39
Sub plot	0.35	1.03	15.76	0.32	0.94	12.86	1.54	4.50	15.07
Interaction S x M	0.5	NS		0.46	1.34		2.18	6.36	

M_1 = Irrigation M_2 = Unirrigation S x M = Sub plot means at fixed level of main plots

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