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IMPACT OF DIFFERENT BIO-STIMULANTS IN *VIGNA UNGUICULATA* UNDER RAINFED CONDITIONS

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SUMMARY

Agricultural biostimulants include diverse formulations of compounds, substances and micro-organisms which applied to plants or soils to improve crop vigour, yields, quality and tolerance of abiotic stresses. The investigation was carried out for the assessment of Physiological responses of various bio-stimulants in *Vigna unguiculata* L. genotype (CS-88) under rainfed condition during the summer season of 2019. The investigation conducted at Dryland Research Farm, Forage Section under rainfed conditions at CCS HAU, Hisar. Bio-stimulants were applied exogenously at flower initiation stage. The physiological parameters in cowpea plant like water relation, gaseous exchange studies chlorophyll content (SPAD units) and photochemical quantum yield showed declining trend in rainfed condition. But with the imposition of different bio-stimulants at flower initiation stage, values of physiological parameters found to be increased. Values ranged from control to biostimulant application in osmotic potential (-MPa) (-1.24 to -1.09), RWC (%) (72.7 - 88.7). Similarly, chlorophyll content (SPAD units) and photochemical quantum yield also showed the increasing trend after foliar application of different biostimulants and the values varied from (41.0 - 51.4) and (0.678 - 0.718), respectively. Reversibly, relative stress injury was found to be decreased from control (35.51) to biostimulants application (20.58) in cowpea under moisture stress. The value of biochemical parameters ranges from proline (131.4 - 381.9 µg g^{-1} DW) and glycine betaine (144.0 - 424.2 µmol g^{-1} DW) over their respective control. Conclusively, based on the above studies it could be concluded that after foliar spray of different biostimulants under rainfed condition, cowpea performed better by maintaining higher plant water status, photosynthetic rate and lower values of RSI (%). Biostimulants treatments not only ameliorate the effect of moisture stress on plants, but also showed a stimulating effect. Application of 2 % complex N, P, K was found more effective which was at par with SA 100 ppm spray at flower initiation than others biostimulants in cowpea.

Key words: Biostimulants, cowpea, Glycine betaine, Proline content and RWC

Cowpea (*Vigna unguiculata* L.) is an annual leguminous plant cultivated primarily for animal fodder and as a food source for humans. It exhibits significant morphological and ecological diversity and is commonly referred to as lobia in various regions. Globally, cowpea is grown on approximately 12.5 million hectares annually, with a total production of around 3.3 million tons of grain (FAO, 2023; Singh *et al*., 2024). In India alone cowpea full-fledged area is about 0.5 m ha with an average production of 600-750 kg/ha. Cowpea is rich in vitamin B, vitamin C (a strong antioxidant) iron, riboflavin and dietary fibre (diarrhea and constipation. The young leaves and undeveloped pods are used as vegetable whereas dried grain and seeds are used to prepare snacks and other dishes. Most cowpea varieties can produce flower and grain for a long period of time. Cowpeas are relatively a drought tolerant and minimum annual rainfall from 300-700 mm is required for reasonable yields. They execute best in warm conditions and an optimum temperature between 20-35°C is fit for its growth. This crop is also used in crop rotation because of its capability to reinstate soil fertility. It can be grown as an intercrop with the maize, pearl millet and sorghum. It can be one of the good alternatives for human beings also due to very high protein content as it is consumed as green pods for vegetable purpose and grains also as cooked meal. Cowpea is considered as an essential produce for sustainable agriculture. Rainfed agriculture in some country of the world has maximum yields. Cowpea shows vast reply with the application of different biostimulants. The application of biostimualnts depends upon the conditions of the soil like moisture content and fertility of the soil. The

effect of biostimulants becomes more consistent with the foliar application than through soil because uptake of nutrients from foliar application is higher than soil. Salicylic acid is the important phenolic compound in the plant growth. The role of salicylic acid in disease resistance is well known. It also enhances enzymatic defense mechanism and decrease damage to cell membrane (Wahid, 2007) improve photosynthetic rate, inhibition of ethylene production (Kumar et al., 2013) increase uptake of N, P, K and other soil minerals thus increase the yield. Foliar application of KCl timely produces more flower, fruit production, increases dry matter deposition, photosynthetic rate, plant height, number of branches, number of root nodule and leaf area. KNO_3 and $CaCl_2$ enhance the yield and improve nutritional quality of the grain. KNO_3 has significant impact on cowpea *i.e*. increase the seed weight and number of leaves. Foliar application of KNO_3 has maximum length of pod, seed weight and increase no. of seed per pod than water sprayed and unsprayed (Sharma et al., 2000). KNO_3 induces translocation of substrate from the vegetative parts to the productive parts of the plants. They act as osmoprotectant to the plant and have various effects on uptake of water, root growth, maintains turgor pressure and thus transpiration in leaves. It also stimulates photosynthesis and cell division (Rasheed et al., 2011) and thus improves the quality of the crop. Increased pod and yield grain by application of $CaCl_2$ might be due to the availability of Ca^{2+}. Application of N and K has better tolerance to photo-oxidative damage and increased photosynthetic capacity. Timely and optimum uses of N, P, K fertilizer not only increase the field of crop but also help to sustain the environment.

MATERIALS AND METHODS

This investigation was carried out to evaluate the effect of foliar application of biostimulants on physiological, biochemical and yield attributes in cowpea genotype under rainfed condition. Seeds were collected from Forage Section, Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar (Haryana) and the experiment was conducted at the Forage Section Research Area of Dryland Research Farm, CCS Haryana Agricultural University, Hisar (Haryana), with the plot size of 2.25m × 5.4 m and 5 lines of 5.4 m length. Sampling was made after 10 days of application of different biostimulants. In the experiment, three replications of the seven treatments viz. T_1: Absolute Control (no spray), T_2: Water spray at flower initiation, T_3: $CaCl_2$ (0.1%) spray at flower initiation, T_4: KCl (0.2%) spray at flower initiation, T_5: KNO_3 (2%) at flower initiation, T_6: Salicylic acid (100 ppm) spray at flower initiation and T_7: 2% Complex N, P, K (19:19:19) spray at flower initiation were laid out in RBD design. Data was analyzed by using one factor OPSTAT software. The means of the above treatments were compared at 5 % level of significance with Online Statistical Analysis Package (OPSTAT, Computer Section, CCS Haryana Agricultural University, Hisar, India)

Plant water status : Relative water content (RWC %) was measured as plants were sampled and third fully expanded leaves was detached from the shoots at mid-day (between 9 AM and 11 AM), quickly sealed in humified polythene bags, and transported to the laboratory on ice (Fig.1.). Leaves were weighed immediately to take their fresh weight and then kept in petridish filled with distilled water for 3 h. After 3-4 hours, the leaf discs were taken out of water and any surface moisture is removed quickly with filter paper lightly and immediately weighed to obtain fully turgid weight (TW). After that the leaves were then kept in an oven at 65°C for 72 h till a constant dry weight. These three weights were used to calculate RWC (%) according to the formula given by Weatherly (1950).

RWC (%) = (Fresh weight – Dry weight) / (Turgid weight – Dry weight) x 100

Biochemical analysis : Proline content was estimated by using the method of Bates et al. (1973). Reagents: 3 % aqueous sulphosalicylic acid (w/v), Acid ninhydrin (prepared by dissolving 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6.0 M o-Phosphoric acid until dissolved), Toluene Extraction: Three hundred mg of fresh leaves were separately homogenized in 5 ml of 3 % sulphosalicylic acid and then centrifuged at 5000 rpm for 15 minutes and supernatant was taken. Procedure: Two ml of supernatant was taken in a test tube and add two ml of ninhydrin reagent. After that two ml of glacial acetic acid was added to the tubes. The test tubes were heated in boiling water bath for 1 hour and thereafter reaction was terminated by keeping tubes in ice-bath immediately. Then 4.0 ml of toluene was added. After vigorous shaking on vortex, the upper organic phase was taken for absorbance. It was recorded at 520 nm by using toluene as blank. A standard curve was prepared by using graded concentration of proline in 3% sulphosalicylic acid. The proline content was expressed as mg g-1DW.

Glycine betaine content (µmol g-1 DW) CSI (%) Glycine betaine was estimated according to the method of Grattan and Grieve (1998). Reagents: 0.05%toluene, 2N HCl, Potassium tri-iodide solution, 1,2-dichloroethane Procedure: Leaves of 500 mg were homogenized separately in 5 ml of 0.05% toluene. All the tubes were kept for 24 h at 25^{0}C. After filtration 0.5 ml of extract was mixed with 1 ml of 2 N HCl solution and 0.1 ml of potassium tri-iodide solution (containing 7.5 g iodine and 10 g potassium iodide in 100 ml of 1 N HCl) was added and shaken in ice cold water bath for 90 min and then 2 ml of ice cold water was added after gentle shaking and then 10 ml of 1, 2-dichloroethane (chilled at -20^{o}C) was pour in it. By passing continuous stream of air for 1-2 minutes two layers were separated, upper aqueous layer was discarded and optical density of organic layer was recorded at 365 nm. Standard curve was prepared using graded concentration of glycine betaine and the data was expressed as mg g^{-1} DW.

Photosynthetic analysis : Chlorophyll content (SPAD units) Chlorophyll content was determined by SPAD 502 plus instrument by measuring the absorbance of the leaf in two wavelength regions (Blue 400-500 nm and Red 600-700 nm). Measurements are taken by simply inserting a leaf and closing the measuring head. Using these two absorbances, the meter calculates a numerical SPAD value which is proportional to the amount of chlorophyll present in leaf. It is not necessary to cut the leaf, so the same leaf can be measured throughout the growing process.

Photochemical quantum yield (Fv/Fm) Chlorophyll fluorescence was recorded in intact plants using chlorophyll fluorometer (OS-30p, Opti-Science, Inc., Hudson, USA) at mid-day. The fully expended leaf was first acclimated to dark for minimum two minutes by fixing clip on it. The dark-adapted leaf was then continuously irradiated for one second (1500 ìmol/m^{-2}/s^{-1}) provided by an array of three light emitted diodes in the sensor. Initial (Fo) and maximum (Fm) fluorescence were recorded and variable fluorescence (Fv), derived by subtracting Fo from Fm. Photochemical quantum yield was then calculated by Fv/Fm ratio.

RESULTS AND DISCUSSION

Plant water status

Osmotic potential of leaf: The values of osmotic potential become less negative with the foliar application of different types of biostimulants as compared to their controlled plants in the cowpea genotype CS-88 at flower initiation stage. The values varied from (-1.24 to -1.08) at the different given treatment of biostimulants. The less negative values were shown by the salicylic acid (100 ppm) followed by treatment 2 % complex N, P, K spray (-1.09), KCl, KNO_3, $CaCl_2$ water spray followed by control (Fig.1). The difference between treatments were significant at flower initiation stage. Relative water content showed increasing trend with the imposition of every biostimulant. Fig. 2. showed the variation in the values of relative water content from control to the treatment (72.7-88.7%). The higher RWC was maintained by the application of SA (100 ppm) followed by 2% complex N, P, K treatment at flower initiation stage. RWC was maintained towards lower side by control followed by water spray and $CaCl_2$. The RWC of KNO_3 was at par with the application of KCl. Interaction between treatments were statistically significant at flower initiation stage.

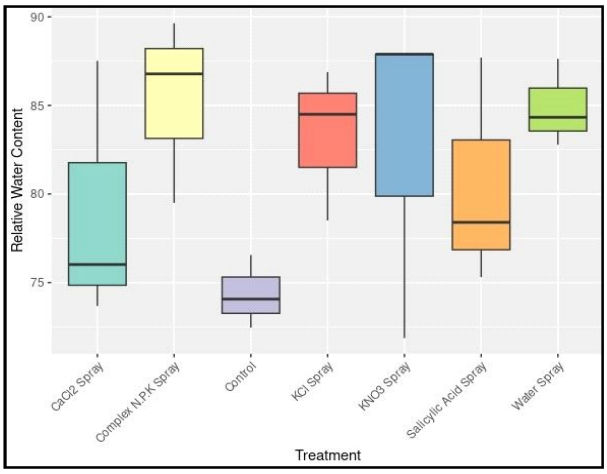

Fig. 1. RWC (%) after foliar spray of biostimulants.

Biochemical analysis: Proline content (µg g^{-1} DW) A significant increment in proline accumulation was noticed with every application of different biostimulants at flower initiation stage (Fig. 2.). Maximum proline content was noticed after foliar application of 2 % complex N, P, K (380.9) followed by 100 ppm salicylic acid (319.6) and KCl (293.6). Foliar application of KNO_3 (267.6) $CaCl_2$ (237.2) and water spray (209.8) enhanced the proline content to a slight extent and a very less amount of proline content was accumulated under control condition with drought stress. Glycine betaine (µmol g^{-1} DW) The glycine betaine content also increased significantly with each foliar application of different biostimulants at flower

initiation stage. Fig.3 showed the variation in the value of proline accumulation and the values varied from (144.0 to 424.1) control to treatment. Highest accumulation in glycine betaine content was noticed with the application of 2 % complex N, P, K (424.1) and lowest in control (144.0).

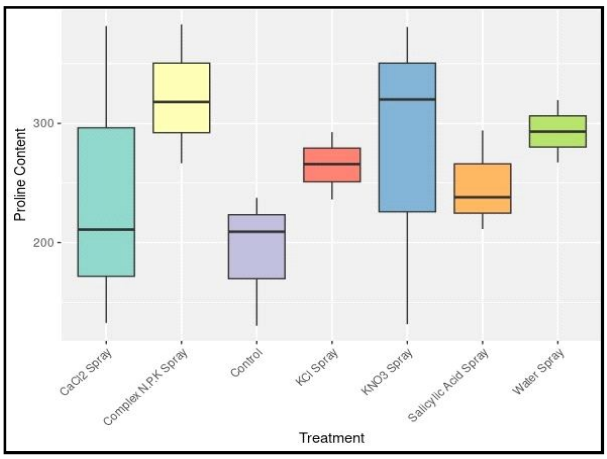

Fig. 2. Proline content after foliar spray of biostimulants at different growth stages.

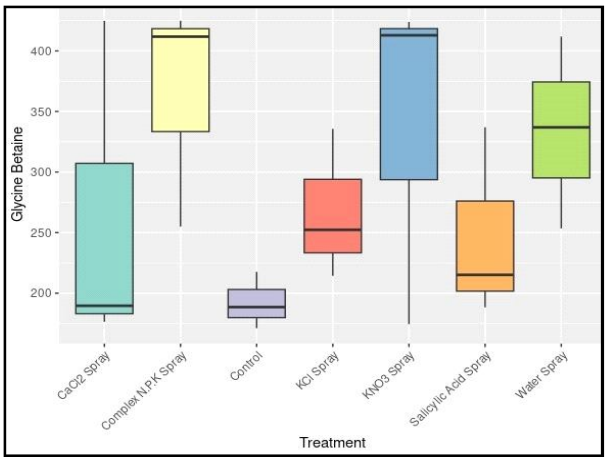

Fig. 3. Glycine betaine content after foliar spray of biostimulants at different growth stages.

Photosynthetic analysis: Chlorophyll content (SPAD units) in terms of SPAD units showed increasing trend with foliar application of different biostimulants (Fig. 4.). Maximum chlorophyll content was noticed in 2% complex N, P, K spray (51.43) followed by SA 100 ppm (51.42) and KCl 0.2% (51.26) at flower initiation stage. The least value was found in the control plants. The chlorophyll content after application of KNO_3 was at par with SA (100ppm) and 2 % complex N, P, K spray at flower initiation. Significant increase was observed in the treatment of 2 % complex NPK spray at flower initiation stage. Generally, photochemical quantum yield (Fv/Fm) increased with the foliar application of different biostimulants (Fig. 5.). The values ranged from (0.678-0.761) control to treatments. The maximum values of Fv/Fm estimated after imposition of 2% complex N, P, K treated plants (0.761) followed by 100 ppm SA (0.718), KCl (0.712) and KNO_3 (0.708) at flower initiation stage. The lowest value was observed in the control (0.678) as compared to the application of other treatments. Significant increase was noticed with each treatment as compare to control.

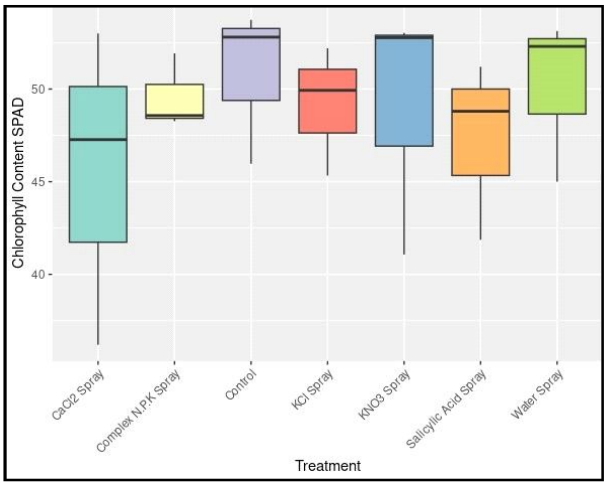

Fig. 4. Chlorophyll content (SPAD units) after foliar spray of different biostimulants.

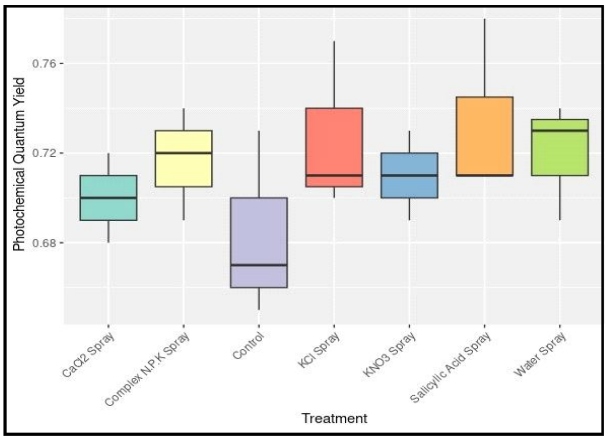

Fig. 5. Photochemical quantum yield after foliar spray of different biostimulants.

CONCLUSION

Conclusively, the foliar application of various biostimulants under rainfed conditions improved cowpea performance by enhancing plant water status, increasing photosynthetic rate and reducing relative stress index (RSI %). These treatments not only mitigated the effects of moisture stress but also exhibited a stimulatory effect on plant growth. Among the treatments, the application of 2% complex NPK

was the most effective, performing on par with a 100 ppm salicylic acid (SA) spray at the flowering stage.

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REFERENCES

El Naim, A.M., A.A. Jabereldar, S.E. Ahmed, F.M. Ismaeil, and E.A. Ibrahim, 2012 : Determination of suitable variety and plants per stand of cowpea (*Vigna unguiculata* L. Walp) in the sandy soil, Sudan. *Advances in life Sciences*, **2**(1): 1-5.

WaI, F.A.O. 2012 : The State of Food Insecurity in the World 2012. Economic growth is necessary but not sufficient to accelerate reduction of hunger and malnutrition. Rome FAO.

Wahid, A., 2007 : Physiological implications of metabolite biosynthesis for net assimilation and heat-stress tolerance of sugarcane (*Saccharum officinarum*) sprouts. *Journal of plant Research*, **120**(2): 219-228.

Kumar, A., P. Kumar, and R. Nadendla, 2013 : A review on: *Abelmoschus esculentus* (okra). *International Research Journal of Pharmaceutical and Applied Sciences*, **3**(4): 129-132.

Sharma, K.K., M. Lavanya, and V. Anjaiah, 2000 : A method for isolation and purification of peanut genomic DNA suitable for analytical applications. *Plant Molecular Biology Reporter*, **18**(4): 393- 393.

Rasheed, R., A. Wahid, M. Farooq, I. Hussain, and S.M. Basra, 2011 : Role of proline and glycine betaine pretreatments in improving heat tolerance of sprouting sugarcane (*Saccharum* sp.) buds. *Plant growth regulation*, **65**(1): 35-45.