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EFFECT OF BIO-STIMULANT APPLICATION ON COWPEA PHYSIOLOGY AND YIELD UNDER SEMI-ARID CONDITION

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SUMMARY

Biostimulants are natural or synthetic substances applied to plants to enhance their growth, development, and stress tolerance without providing essential nutrients. Their use in agriculture has gained significant attention as a sustainable strategy to boost crop productivity, enhance quality, and reduce dependency on chemical fertilizers and pesticides. With growing concerns over climate change and resource limitations, biostimulants offer an eco-friendly solution to optimize crop performance and improve resilience in challenging environments. This study was conducted at the Dryland Research Farm, Forage Section, CCS HAU, Hisar, to evaluate the physiological effects of various bio-stimulants on the cowpea genotype CS-88 (*Vigna unguiculata* L.) under rainfed summer conditions in 2019. Specifically, the experiment aimed to assess how these bio-stimulants influenced the plant responses when applied exogenously at the flower initiation stage. The physiological parameters in cowpea plant like gaseous exchange studies chlorophyll content (SPAD units) and photochemical quantum yield showed declining trend in rainfed condition**.** Values ranged from control to biostimulant application in assimilation rate (23.31 - 29.73 µmol CO_2 m^{-2} s^{-1}), transpiration rate (1.70 - 2.07 mmol H_2O m^{-2} s^{-1}) and stomatal conductance (0.02 - 0.03 mmol H_2O m^{-2} s^{-1}). 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. Soil analysis was also done to find the best response of these biostimulants.

Key words: Biostimulants, cowpea, glycine betaine, proline content and RWC

Cowpea (*Vigna unguiculata* L.), a vital legume globally, is cultivated for both human consumption and animal feed, particularly in arid and semi-arid regions. It is considered as one of the oldest legume used as protein source for humans and livestock (Panchta *et al.*, 2020). It is typically adapted to biotic and abiotic stresses like drought, high temperatures, and other factors better than other pulses (Ankita *et al.*, 2023). Globally, cowpea is grown on approximately 14.5 mha, producing over 7 mt annually, reflecting its growing importance in food security (FAOSTAT, 2023). In India, cowpea cultivation covers around 0.5 million hectares, with average yields ranging from 600-750 kg/ha, indicating potential for yield enhancement through improved agronomic practices and stress-tolerant varieties (Anonymous, 2023). To address these challenges, foliar application of nutrients and growth regulators has been identified as an effective strategy to enhance growth, flowering, and yield (Pereira *et al.*, 2022; Sharma *et al.*, 2021). Cowpea's nutritional profile is rich, providing essential vitamins (B and C), minerals (iron, riboflavin), and dietary fiber, contributing to human health (Langyintuo *et al.*, 2019). Its adaptability to drought, requiring minimal rainfall (300-700 mm) and thriving in warm climates (20-35°C), makes it a crucial crop for sustainable agriculture and climate resilience (Timko *et al.*, 2022). Cowpea's ability to fix atmospheric nitrogen also enhances soil fertility, making it valuable for crop rotation and intercropping with cereals like maize, pearl millet, and sorghum (Ajeigbe *et al.*, 2020).

Recent studies have evaluated various foliar treatments to improve cowpea performance. For example, the application of calcium chloride ($CaCl_2$) has been shown to improve flowering and pod set by stabilizing cell membranes and promoting nutrient uptake (Singh *et al.*, 2020; Agboola *et al.*, 2021). Similarly, potassium chloride (KCl) has been found to enhance plant growth and yield under drought stress by improving osmotic adjustment and maintaining cellular integrity (Ahmed et al., 2021). Nitrogen-based foliar sprays, such as potassium nitrate (KNO_3), have also been reported to improve the efficiency of nitrogen metabolism and photosynthesis, thereby increasing cowpea productivity (Ravindra *et al.*, 2020; Sharma *et al.*, 2019).

Salicylic acid (SA), a plant growth regulator, has garnered attention for its role in enhancing stress tolerance and promoting metabolic processes during flowering stages, leading to increased pod and seed yield in cowpea (Saha *et al.*, 2021; Liu *et al.*, 2020). Additionally, the use of complex fertilizers like NPK (19:19:19) has been shown to improve nutrient availability and uptake, resulting in improved growth and yield, particularly under nutrient-deficient conditions (Kumar *et al.*, 2022; Rizvi *et al.*, 2021).

These studies collectively suggest that foliar application of bio-stimulants and nutrients can significantly improve physiological responses of cowpea, especially under rainfed conditions. However, the effectiveness of such treatments varies depending on factors such as the type of bio-stimulant, nutrient formulation, and environmental stressors. Therefore, further research is required to optimize application timing and concentration for sustainable cowpea cultivation under water-limited conditions.

MATERIALS AND METHODS

This investigation was carried out to evaluate the effect of foliar application of biostimulants on physiological and yield attributes in cowpea variety (CS 88) under rainfed condition. Seeds were collected from Forage Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar (Haryana) and the experiment was conducted at the Dryland Research Farm, CCS Haryana Agricultural University, Hisar (Haryana), with the plot size of 2.25 m × 5.40 m and 5 lines of 5.4 m length. The soil of the experimental field was sandy loam in texture, slightly alkaline in reaction (pH 7.5), low in available nitrogen (128.5 kg/ha), medium in available phosphorus (11.0 kg/ha) and medium in potassium (195.5 kg/ha). In this field trial, 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, T_7: 2% Complex NPK (19:19:19) spray at flower initiation, were replicated thrice in RBD design. Sampling was made after 10 days of application of different biostimulants. The statistical analysis of the data was carried out by using one factor SAS 9.3 programme.

Gaseous exchange parameters: Photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$), Transpiration rate (mmol H_2O m^{-2} s^{-1}) and stomatal conductance (mmol H_2O m^{-2} s^{-1}) of fully expended leaf were measured by infrared gas analyzer (IRGA LCi-SD, ADC Biosciences). The leaf was enclosed in the assimilation chamber and position was shifted such that maximum PAR was obtained then Photosynthetic rate (A), Transpiration rate (E) and stomatal conductance (gs) was monitored while CO_2 concentration changed over a definite time interval. The system automatically calculated the A, E and gs on the basis of preloaded flow rate and leaf area. Measurements were taken at 10:00 to 11:00 h (Indian standard time). When relative humidity, temperature, photosynthetic photon flux density and amp; CO_2 concentration ranged from 50–60%, 25–35°C, 1200 µmole (photon) m^{-1} s^{-1} and amp; 350–360 ìmole^{-1}, respectively.

Photosynthetic pigments: 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) or 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.

Yield and yield attributes

Seed yield per plant (g): Seeds of each plant was collected from the individual plant and weighted on electronic balance. 100 seed weight was taken by counting the seeds and then weighted on electrical balance. Harvest index is represented in terms of percentage. The harvest index for each plot was calculated by dividing the economic (grain) yield from biological yield (seed + stover yield) of the same net plot and multiplied by100 as given below:

HI = (Economic yield / Biological yield) x 100

Soil analysis: Soil samples were taken from different spots of the experimental field before sowing and were analyzed for soil texture, and EC, pH, N, P and K contents as per the method given in Table 1 and 2.

TABLE 1
Mechanical analysis of the soil of dry land experimental area before sowing

Components	Values	Estimation method
Sand (%)	82.3	International pipette method (Piper, 1966)
Silt (%)	10.2	
Clay (%)	7.6	

RESULTS AND DISCUSSION

Photosynthetic pigments:

Chlorophyll content (SPAD units) in terms of SPAD units showed increasing trend with foliar application of different biostimulants. 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 (Fig.1). 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. 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 (Fig.2). 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.

Gas exchange parameters: It is observed from the Fig. 3 that assimilation rate gradually increases with the application of different biostimulants. Values of assimilation rate varied within the range (23.3- 27.5 μmol CO_2 m^{-2} s^{-1}) in cowpea under moisture stress. Under rainfed conditions assimilation rate decreases and enhanced with the foliar application of biostimulants with a maximum value in SA treated plants (29.7) which was at par with 2% complex N, P, K treatment (27.5). Minimum assimilation rate was noticed under control conditions (23.3) at flower initiation stage. Other treatments *i.e.* water spray (24.7), $CaCl_2$ (25.7), KNO_3 (26.4) and KCl (27.1) also showed the enhanced assimilation rate as compared to control. Significant increase between each treatment was noticed at flower initiation stage. Transpiration rate decreased with the onset of moisture stress but increased with the foliar application of different biostimulants at flower initiation stage. Transpiration rate is directly related with the water loss in plants through stomata. The highest transpiration rate observed with the imposition of SA 100 ppm treatment

TABLE 2
Chemical composition of soil of dry land experimental area before sowing

S. No.	Particulars	Results	Category	Method used
1.	Soil texture	Sandy loam	Sandy loam	Bouyoucos hydrometer method (Piper *et. al.*, 1966)
2.	Soil pH value	7.5	Slightly alkaline in reaction	Determined in soil : distilled water suspension (1:2) (Jackson *et. al.*, 1973)
3.	EC (dS/m)	0.33	Non saline	
5.	Available Nitrogen (kg/ha)	128.5	Low	Alkaline permanganate method (Subbiah *et. al.*, 1956)
6.	Available Phosphorus (kg/ha)	11.5	Medium	Sodium bicarbonate method (Olsen *et. al.*, 1954)
7.	Available Potassium (kg/ha)	195.5	Medium	Neutral normal ammonium acetate method (Jackson *et. al.*, 1973)

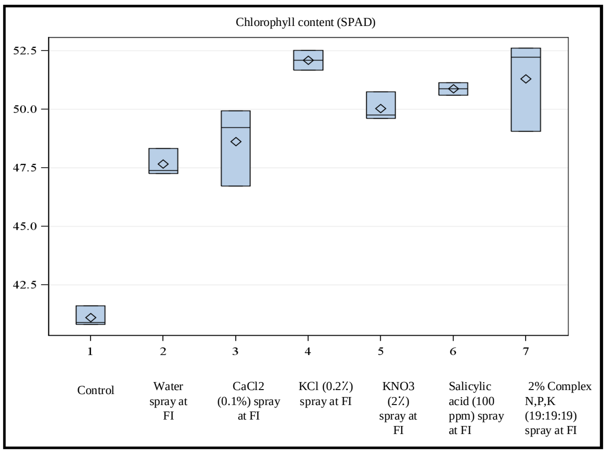

Fig. 1. Chlorophyll content (SPAD) in cowpea variety after foliar spray of biostimulants.

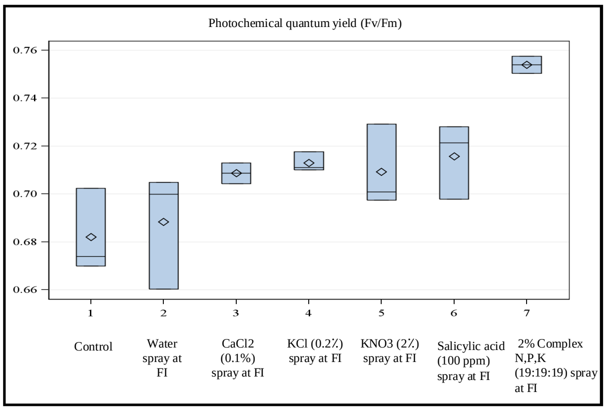

Fig. 2. Photochemical quantum yield (Fv/Fm) in cowpea variety after foliar spray of biostimulants.

followed by 2 % complex NPK treatment (2.08 mmol H_2O $m^{-2}s^{-1}$) and KCl 0.2 % foliar spray at flower initiation Fig.4. Treatments were statistically significant at flower initiation stage. It is revealed from the Fig. 5 that stomatal conductance decreased with the imposition of moisture stress. Foliar application of different biostimulants increased the stomatal conductance under rainfed condition in cowpea. Highest value of stomatal conductance was observed after the imposition of 100 ppm SA (0.031) followed by 2% complex NPK (0.028) and KCl 0.2% spray (0.024) at flower initiation. Treatments were noticed significant at flower initiation stage.

Yield and yield attributes

Seed yield per plant also shows the increasing trend with the application of different biostimulants. Maximum seed yield was calculated with the implementation of 2% complex NPK (1.64) followed by 100 ppm SA spray (1.48) and KCl (1.39) in comparison to the control at flower initiation stage Fig.6. Significant increase in values was noticed at flower initiation stage. It is depicted from the Fig.7 that there is a gradual increase in the 100 seed weight in cowpea with the foliar application of biostimulants under rainfed conditions. Maximum seed weight was calculated after foliar spray of 2% complex NPK (10.21) at flower initiation stage. Significant increment in values was noticed at flower initiation stage. Results presented in Fig.8 depict a gradual increase in harvest index with the spray of different biostimulants. Highest harvest index was calculated after foliar application of KCl @ 0.2% (0.59) and 2% complex NPK 19-19-19 (0.59) at flower initiation stage in comparison to the control plant.

Discussion: Fig. (3, 4 and 5) shows the decrease in the gaseous exchange under moisture stress and after the application of biostimulants gas exchange parameters increases in cowpea plant. Photosynthesis under rainfed is readily affected by stomatal conductance, as closure of stomata checks the CO_2 diffusion from chloroplast hence declining the internal CO_2 concentration (Cornic, 2000) and affects the enzyme activity such as Rubisco (Maroco *et al*., 2002). In this study, cowpea plant showed in Fig. (6, 7 and 8) indicated decrease of yield parameters under drought stress but with the application of different biostimulants at different level of concentration showed improvement in yield parameters.

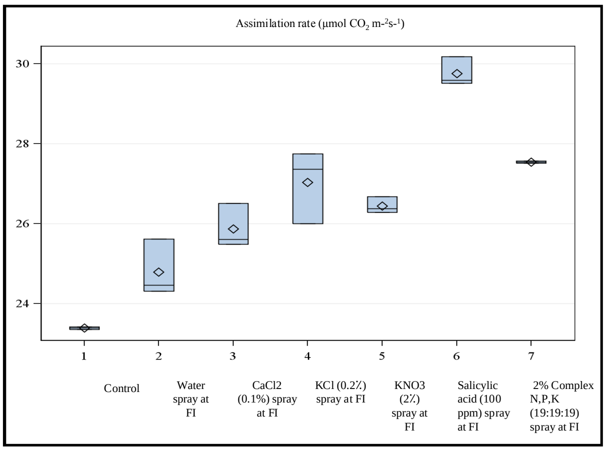

Fig. 3. Assimilation rate (µmol CO_2 $m^{-2}s^{-1}$) in cowpea variety after foliar spray of biostimulants.

CONCLUSION

Moisture stress showed adverse effect on yield and yield attributes, so seed yield per plant, 100 seed weight and harvest index decreased under

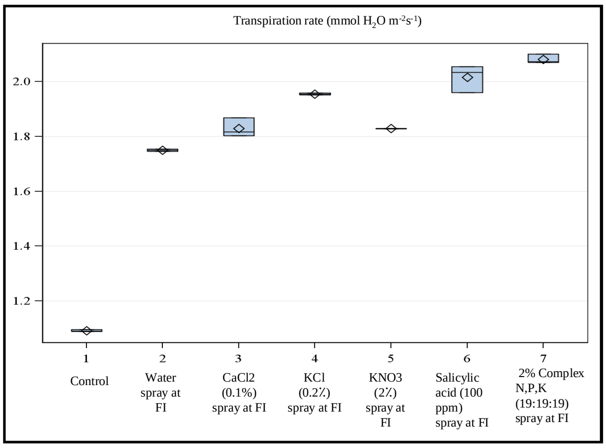

Fig. 4. Transpiration rate (mmol H_2O $m^{-2}s^{-1}$) in cowpea variety after foliar spray of biostimulants.

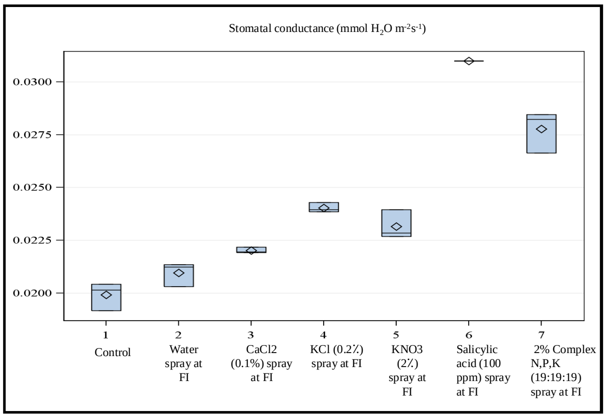

Fig. 5. Stomatal conductance (mmol H_2O $m^{-2}s^{-1}$) in cowpea variety after foliar spray of biostimulants.

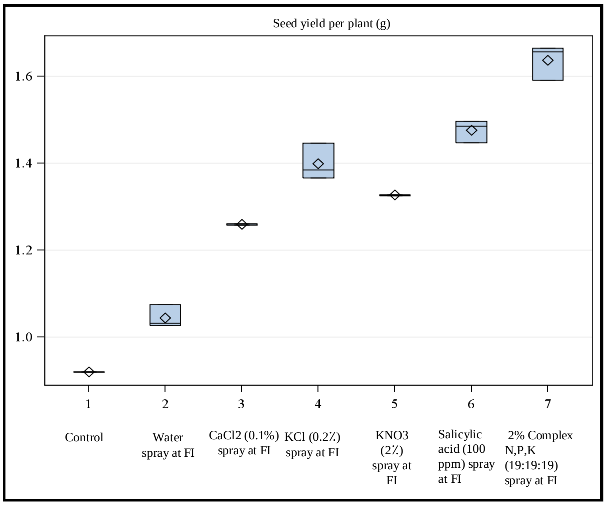

Fig. 6. Seed yield per plant (g) in cowpea variety foliar spray of biostimulants.

moisture stress in cowpea. Maximum yield attributes were observed after foliar spray of 2% complex NPK at flower initiation stage in cowpea. Significant increment in values was noticed at flower initiation stage in various treatments as compare to control.

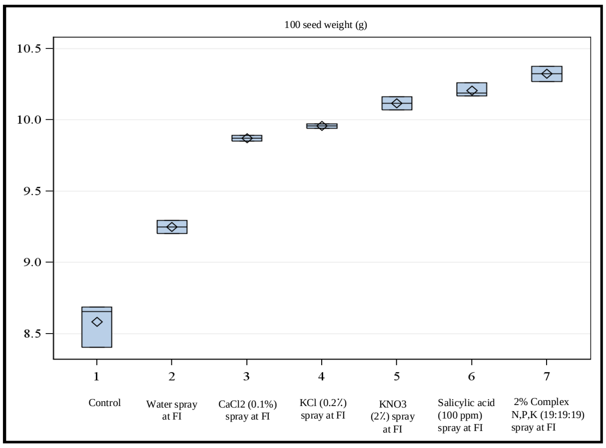

Fig. 7. 100 seed weight (g) in cowpea variety after foliar spray of biostimulants.

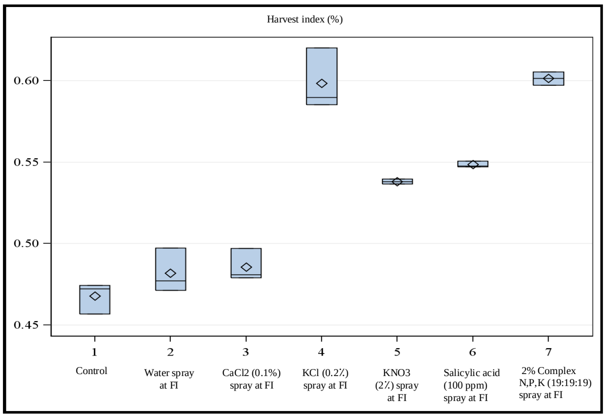

Fig. 8. Harvest index (%) in cowpea variety foliar spray of biostimulants.

Chlorophyll stability index, chlorophyll content (SPAD units) and photochemical quantum yield significantly decreased under rainfed condition. Maximum values of above parameters were noticed in 2% complex NPK spray followed by salicylic acid @ 100 ppm and KCl 0.2% at flower initiation stage. The least value was found in the control cowpea crop. Decrease in gaseous exchange *i.e.* assimilation rate, transpiration rate and stomatal conductance of leaves was estimated under moisture stress in cowpea. Foliar application of different biostimulants increases the values of above parameters under rainfed condition in cowpea. Cowpea plants performed better after the imposition of 100 ppm SA or 2% complex NPK (19:19:19) at flower initiation stage under moisture stress conditions.

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