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Full Length Article

Exogenous Application of Bio-regulators Improves Grain Yield and Nutritional Quality of Forage Cowpea (*Vigna unguiculata*)

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Abstract

This study was conducted at Fodder Farm, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India for two consecutive years (2008 and 2009) to investigate the effect of foliar application of bio-regulators applied at different concentrations *viz.* sodium benzoate (100 and 150 µg mL^{-1}), salicylic acid (50 and 100 µg mL^{-1}), $CaCl_2$ (0.5 and 1.0%) and KNO_3 (1.0 and 2.0%) on grain yield and quality of two forage cowpea cultivars CL 367 and Cowpea 88. Three foliar sprays of each bio-regulators were applied at weekly interval with the onset of flower initiation. Forage cowpea cultivar CL 367 exhibited significantly (p = 0.05) higher biological, pod, grain and crude protein yield than cultivar Cowpea 88. Neutral detergent fiber (NDF), hemicellulose and organic matter content of grain were also higher in cultivar CL 367. Phosphorus (P), potassium (K), zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) content were significantly better in Cowpea 88 cultivar. Foliar application of different bio-regulators increased the grain yield of cowpea by 12.9-32.7%. The highest grain and crude protein yield was recorded in foliar application of 50 µg mL^{-1} salicylic acid treatment. Grains produced under 50 µg mL^{-1} salicylic acid treatment had better quality in terms of more N, P, K, Zn, Cu, Fe, crude protein and organic matter content than the control. Also significant reduction in ether extract, NDF, hemicellulose and ash content of grain were recorded with foliar application of 50 µg mL^{-1} salicylic acid. Foliar application of KNO_3 and $CaCl_2$ also improved the yield and nutritional quality of the grain. The increased seed yield and quality of cowpea with bio-regulators certainly will improve the livestock health and milk production thereby improving the income of the farmers in South-East Asia and other tropical countries.

Keywords: Bio-regulators; Cowpea; KNO_3; Salicylic acid; Seed yield; Quality

Introduction

Heat stress due to increased global atmospheric temperature could be one of the major constraints for limiting crop yield and quality in many crops (Wahid *et al.,* 2007). Studies indicated that heat stress accompanied by low soil moisture during flowering and pod setting stage in cowpea producing areas limit the grain yield and quality mainly due to reduced seed filling rate or duration and little seed set and weight (Mutters and Hall, 1992; Wahid *et al.,* 2007). Heat stress can have profound impact on seed quality of cereals and legumes, mainly because of their impact on nutrient uptake, assimilate supply and partitioning, and reduced carbohydrate and protein accumulation in grain (Thuzar *et al.,* 2010; Devi *et al.,* 2011; Kumar *et al.,* 2013). With the passage of time, climate change is likely to accentuate the frequency and intensity of heat and drought stresses, which definitely will affect the grain yield and quality of crops. High night temperature above 20°C particularly during flowering in cowpea induces male sterility (Ahmed and Hall, 1993), which causes loss in grain yield and quality due to poor and shriveled pod setting (Ahmed and Hall, 1993; Hayat *et al.,* 2010; Mahmood *et al.,* 2010). High night temperature at flowering also stimulate respiration, damage the cell membranes and reduces net photosynthesis (Kumar *et al.,* 2013) which affects the transfer of photo-assimilates into the seed and nutrient uptake by the roots from soil (Wahid *et al.,* 2007).

Bio-regulators use is an emerging plant biotechnology approach which can modify plant gene expression, can affect levels of DNA, RNA, enzymes and finally their products such as protein, carbohydrates, lipids, allelochemicals for enhancing yield and phytonutrients in food crops (Olaiya *et al.,* 2013). A high night temperature during growth, flower initiation and reproductive phase in cowpea from June-September during summer season in

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North-West India remains around 24°C (Prabhjyot-Kaur *et al.,* 2006), which adversely affects the flowering and seed setting in cowpea thereby reducing its yield and quality.

Heat and drought stress in field crops can be managed by applying bio-regulators like sodium benzoate, salicylic acid, calcium chloride and potassium nitrate, which are able to induce long term thermo-tolerance in plants and can be helpful in mitigating the yield reduction threats as well as are helpful in producing good quality grains (Wahid *et al.,* 2007; Kumar *et al.,* 2013). Salicylic acid retards ethylene synthesis, stimulate photosynthetic machinery, increases the chlorophyll content and is reported to increase grain yield in cereals and legumes under high temperature stress condition (Hayat *et al.,* 2010; Kumar *et al.,* 2013). Sodium benzoate is efficient hydroxyl radical scavenger which protects the plant against oxidative damage under stress condition by inhibiting the ethylene synthesis and is reported to increase the seed yield of crops (Beltrano *et al.,* 1999; Kumar *et al.,* 2013). KNO_3 and $CaCl_2$ acts as osmo-protectants and have profound effect on the crop growth through their effects on water uptake, root growth, maintenance of turgor and transpiration in leaves. Osmo-protectants also stimulate photosynthetic machinery and cell division (El-Abou, 2011; Rasheed *et al.,* 2011) and hence improve the yield and quality of crops. But, there is a lack of comprehensive information available in the literature on the effect of different bio-regulators on grain yield and its nutritional quality in forage cowpea.

The chemical modification of plants by bio-regulator application at low concentrations has the potential to increase food production and quality much more quickly than plant breeding techniques (Wahid *et al.,* 2007; Rasheed *et al.,* 2011). The present study was carried out to reveal the comparative influences of salicylic acid, sodium benzoate, potassium nitrate and calcium chloride on grain yield and quality of two cultivars of fodder cowpea varying in their flowering behavior in north-western India.

Materials and Methods

Site Description and Experimental Design

Field experiments were performed during summer seasons of 2008 and 2009 at the Fodder Farm, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India (30°56′N, 75°52E′; 247 m above sea level). The experiment was laid out in a factorial randomized complete block design with three replications. The treatments included combinations of two cultivars (CL 367 and Cowpea 88) of forage cowpea, and 4 combinations of bio-regulators and their concentrations namely, sodium benzoate (100 and 150 µg mL^{-1}), salicylic acid (50 and 100 µg mL^{-1}), $CaCl_2$ (0.5 and 1.0%) and KNO_3 (1.0 and 2.0%). An additional untreated control was also included in the study. Cultivars were taken as Factor A whereas various bio-regulators as Factor B. The flower initiation in cultivar Cowpea 88 was 5-7 days earlier than cultivar CL 367 during both the years. During the year 2008, flower initiation in cultivar Cowpea 88 started on September 18, whereas in cultivar CL 367 flowering started from September 24. The corresponding dates for flower initiation in cultivar Cowpea 88 and CL 367 during the year 2009 were September 21 and September 26, respectively. Three foliar sprays of the bio-regulators were applied at weekly interval starting from flower initiation in both the cultivars.

Climate and Soil Characteristics

The climate of experimental site is characterized as sub-tropical and semi-arid with hot and dry summer from April to June, hot and humid from July to September and cold winter from November to January. The average annual rainfall is about 705 mm, mostly received during the monsoon period from July to September. A few showers are also received during winter season in months of December and January. The meteorological data were recorded at meteorological observatory of Punjab Agricultural University, Ludhiana. Total amount of precipitation received during 2008 and 2009 crop seasons were 424.1 and 213.4 mm, respectively. Mean maximum and minimum monthly air temperatures recorded were 34.3°C and 12.6°C during the month of August and November and 34.7°C and 11.3°C in September and October months during the year 2008 and 2009, respectively. Minimum temperature during flowering and reproductive phase (38^{th} and 39^{th} standard week) of crop was 20.9°C and 21.9°C and 24.3°C and 24.7°C during the second week to end of September during the year 2008 and 2009, respectively. The soil of the experimental field was sandy loam in texture having pH 7.2, with 0.25% Walkley and Black organic carbon (Low in % OC), 11.3 kg P ha^{-1} Olsen P (medium in available P) and high in available K as 289 kg K ha^{-1} NH_4OAc-extractable K.

Crop Management

The cowpea crop was sown on August 7 and 8 during the year 2008 and 2009, respectively in lines keeping the row to row distance as 30 cm in well prepared seed bed using 20 kg seed ha^{-1} for cultivar CL 367 and 40 kg seed ha^{-1} for cultivar Cowpea 88. Seeds of both the cultivars were treated with Bavistin (carbendazim) at 2 g kg^{-1} of seed before sowing for protecting the crop from fungal diseases. A basal dose of 20 kg N ha^{-1} (as urea) and 24 kg P ha^{-1} (as single super phosphate) were applied at sowing. Stomp (Pendimethalin) 30 EC at 2 L ha^{-1} was applied immediately after sowing of the crop during both the year to control the weeds. Total of four irrigations during 2008 and five irrigations during 2009 were applied to the crop during the growing seasons. For controlling the aphid and jassid during the crop growth, Malathion spray at 500 mL ha^{-1} was done on September 14 during 2008 and September 19 during 2009, respectively. The seed yield was taken on November 11, 2008 and November 13, 2009 when the pods turned yellow.

Recorded Traits

Biological, pod and grain yield: After harvesting of the crop from 15 m^2 area, the produce was left in the field for one week and when leaves and pods gets completely dried, the produce was weighed as biological yield and converted to t ha^{-1}. Pods from the plants in each plot were picked, weighed and converted to pod yield (t ha^{-1}). By beating with wooden sticks manually, grains were separated, cleaned and sun dried thoroughly. A sub-sample of pod and grain was collected and oven dried at 60°C for 48 h to determine the moisture content. Pod and grain yields were computed on oven-dry weight basis.

Laboratory studies: The grains from each treatment plots were collected and subjected to proximate analysis and for macro and micronutrient nutritive quality estimation.

Proximate analysis of the grain: Percentage N and Ether Extract (EE) content in grain was estimated by AOAC (2000). Crude protein (CP) content in grains on oven dry weight basis was worked out by the formula % CP = %N × 6.25. Crude protein yield was worked out by multiplying the crude protein percentage with grain yield and then dividing it by 100. Neutral and acid detergent fibers (NDF and ADF) were estimated according to the method of Van Soest *et al.* (1991). Cellulose content was calculated by difference between ADF and acid detergent lignin (ADL) and hemicellulose (Hem) by difference between NDF and ADF. Total ash content was measured by complete ignition of the sample at 600°C in muffle furnace with the formula weight of ash/weight of sample × 100. Organic matter (OM) was calculated as 100 - % ash.

Grain nutritive quality: The micronutrient contents *viz.* Zn, Cu, Fe and Mn of harvested grains as per treatments were estimated by Atomic Absorption Spectrophotometer (Varian AAS FS 240 Model). Percent phosphorus (P) and potassium (K) in cowpea grains were estimated by the methods described by Jackson (1973).

Statistical Analysis

The data were analyzed using analysis of variance (ANOVA) by using IRRISTAT version 92 (IRRI, 1992). The crop data were analyzed keeping varieties as factor A and foliar treatments as factor B in factorial randomized block design. The data presented is averaged over the years and comparison of treatment means was made by least significant difference (LSD) at $p = 0.05$.

Results

No significant ($p = 0.05$) cultivar × bio-regulator treatment interactions were observed in yield parameters and grain quality of forage cowpea in two years of the study. Therefore, effects of cultivar and foliar treatments are presented and discussed. Similarly, there was no significant treatment × year interaction on different parameters, and only effects of years have been presented in Table 4.

Yield Parameters

Yield is the manifestation of various morphological, physiological and growth parameters in any crop. Forage cowpea cultivar CL 367 recorded significantly higher biological, pod, grain and crude protein yield, than the cultivar Cowpea 88 (Table 1). The pod and grain yield in cultivar CL 367 was 36.0 and 37.6% higher than Cowpea 88, respectively. The data averaged over the years showed that CL 367 cultivar recorded significantly higher biological yield (by 6.0%) and crude protein yield (by 37.6%) over cultivar Cowpea 88.

Foliar spray of bio-regulators indicated significant effect on biological, pod, grain and crude protein yield of cowpea. The highest pod, grain and crude protein yield were recorded in foliar application of salicylic acid at 50 µg mL^{-1} and was statistically at par with 2% KNO_3 and 1% $CaCl_2$ and significantly better than other bio-regulators and control (Table 1). Salicylic acid at 50 µg mL^{-1} recorded 32.5% and 32.7% higher pod and grain yield than control. Foliar application of 2% KNO_3 and 1% $CaCl_2$ recorded 26.7% and 23.5% in pod yield and 27.5% and 24.8% increase in grain yield over control. All bio-regulators recorded higher biological yield than control and were found statistically at par ($p = 0.05$) with salicylic acid at 50 µg mL^{-1}. Foliar application of salicylic acid recorded the highest crude protein yield (4.0 q ha^{-1}) followed by 2% KNO_3 (3.74 q ha^{-1}) and were significantly better than sodium benzoate (100 and 150 µg mL^{-1}), 0.5% $CaCl_2$ and 1% KNO_3.

Foliar applications of sodium benzoate exerted least effect on pod and grain yield in our study but both the traits were found significantly better than control. Mean increase in grain yield was 15.3% and 19.0%, respectively, at the application rate of 100 µg mL^{-1} and 150 µg mL^{-1} of sodium benzoate and were found to be significantly better than untreated control. Application of salicylic acid at 100 µg mL^{-1} significantly reduced the pod and grain yield as compared to salicylic acid at 50 µg mL^{-1} (Table 1).

Proximate Analysis of Grain

Different cultivars did not vary significantly ($p = 0.05$) for N and crude protein percentage in grain (Table 1 and 2). Neutral detergent fiber (NDF) was 4.2% higher in cultivar CL 367 than Cowpea 88 (Table 3). There was no significant difference in fat (ether extract) and acid detergent fiber (%) in different cultivars. Cellulose content of cultivar CL 367 was significantly less than Cowpea 88, whereas hemi cellulose content followed reverse trend (Table 3). Grains of cultivar CL 367 recorded significantly lesser ash content and higher organic matter content which is desirable trait for improvement in the quality of grain.

Foliar application of bio-regulators had significant effect on N, crude protein content, crude protein yield, ether extract, NDF, hemicellulose, ash and organic matter content of cowpea grain (Tables 1-3).

Table 1: Effect of different bio-regulators on biological yield, pod yield, seed yield, crude protein (%) and crude protein yield of forage cowpea (data averaged over two years)

	Biological yield (q ha^{-1})	Pod yield (q ha^{-1})	Grain yield (q ha^{-1})	Crude protein (%)	Crude protein yield (q ha^{-1})
Cultivars					
CL 367	104.5a	21.4a	16.1a	24.4	3.95a
Cowpea 88	99.4b	15.8b	11.7b	24.4	2.87b
LSD ($p = 0.05$)	3.5	0.6	0.4	NS	0.12
Bio-regulators					
Control	83.1d	15.7d	11.6d	22.8c	2.66d
Sodium benzoate (100 µg mL^{-1})	96.5c	17.3c	13.1c	23.6bc	3.08c
Sodium benzoate (150 µg mL^{-1})	100.3bc	18.1bc	13.8bc	23.9b	3.31c
Salicylic acid (50 µg mL^{-1})	112.0a	20.8a	15.5a	25.9a	4.00a
Salicylic acid (100 µg mL^{-1})	105.9ab	19.0b	14.2b	24.5b	3.50bc
$CaCl_2$ (0.5%)	104.2b	18.5c	13.9bc	24.5b	3.43bc
$CaCl_2$ (1.0%)	106.0ab	19.4ab	14.7abc	24.9ab	3.65b
KNO_3 (1.0%)	103.6bc	19.0b	13.9b	24.1b	3.33c
KNO_3 (2.0%)	107.3ab	19.6ab	14.8ab	25.4ab	3.74ab
LSD ($p = 0.05$)	7.5	1.4	0.9	1.0	0.26

Table 2: Effect of different bio-regulators on nutritive quality of cowpea grains (data averaged over two years)

	N	P	K	Zn	Cu	Fe	Mn
	(%)			(mg kg^{-1})			
Cultivars							
CL 367	3.91	0.55b	1.28b	34.7b	2.65b	33.6b	12.6b
Cowpea 88	3.90	0.67a	1.63a	39.6a	3.71a	36.2a	14.2a
LSD ($p = 0.05$)	NS	0.01	0.03	1.13	0.12	1.02	0.39
Bio-regulators							
Control	3.65c	0.58c	1.37c	34.3c	2.76c	33.1b	12.7
Sodium benzoate (100 µg mL^{-1})	3.76c	0.60bc	1.42bc	36.9b	3.15b	33.9b	13.3
Sodium benzoate (150 µg mL^{-1})	3.82bc	0.61b	1.44b	37.0b	3.38ab	34.7b	13.3
Salicylic acid (50 µg mL^{-1})	4.14a	0.64a	1.51a	39.5a	3.45a	38.0a	13.8
Salicylic acid (100 µg mL^{-1})	3.93b	0.58c	1.45b	37.0b	2.96bc	36.6ab	13.5
$CaCl_2$ (0.5%)	3.94b	0.62ab	1.44b	38.5ab	3.17b	34.7b	13.6
$CaCl_2$ (1.0%)	3.97b	0.63ab	1.48ab	38.6ab	3.30ab	35.0b	13.5
KNO_3 (1.0%)	3.85bc	0.61b	1.47ab	35.1bc	3.22ab	33.8b	13.1
KNO_3 (2.0%)	4.06ab	0.63ab	1.50ab	37.7ab	3.30ab	35.9ab	13.8
LSD ($p = 0.05$)	0.16	0.02	0.05	2.4	0.25	2.2	NS

Means sharing the same letter in a column do not differ significantly at $p = 0.05$; NS= Non significant

Highest N, crude protein content and crude protein yield were recorded in foliar application of 50 µg mL^{-1} of salicylic acid followed by 2% KNO_3 and 1% $CaCl_2$ and was significantly ($p = 0.05$) better than control. Crude protein content varied from 22.8% in control to 25.9% in foliar application of 50 µg mL^{-1} of salicylic acid and was statistically at par with foliar applications of 2% KNO_3 and 1% $CaCl_2$. Salicylic acid at 50 µg mL^{-1} recorded significantly less ether extract and NDF content in grain than all other foliar treatments and control. Different bio-regulators fail to exert any significant effect on ADF and cellulose content, however hemi cellulose content was significantly low in 50 µg mL^{-1} of salicylic acid foliar application and was statistically at par with 2% KNO_3. The highest organic matter content and lowest ash content of grain were also recorded in salicylic acid at 50 µg mL^{-1} treatment (Table 3).

Grain Nutritive Quality

Nutritive quality of grain was found to be affected by different cowpeas cultivars in our study. Cultivar Cowpea 88 manifested significantly higher P, K, Zn, Cu, Fe and Mn content in grain than cultivar CL 367 (Table 2). Grains of cultivar Cowpea 88 recorded 21.8% P and 27.3% K more than CL 367 cultivar. Cultivar Cowpea 88 recorded 14.1, 40, 7.7 and 2.7% more Zn, Cu, Fe and Mn content in the grain than cultivar CL 367.

Application of different bio-regulators had shown positive effect on macro- and micronutrient content of cowpea grain in our study. Foliar application of 50 µg mL^{-1} salicylic acid recorded maximum increase in P (10.3%), K (10.2%), Zn (15.1%), Cu (25%), Fe (16.3%) and Mn (8.6%) content of grain than control (Table 2). Higher concentrations of bio-regulators such as sodium benzoate, $CaCl_2$ and KNO_3 improved the micronutrient content of grain than lower concentration but the differences were non-significant ($p = 0.05$). Higher concentration of salicylic acid (100 µg mL^{-1}) had significantly reduced zinc and copper content of grain than foliar application of 50 µg mL^{-1} (Table 2).

Table 3: Effect of different bio-regulators on proximate analysis of cowpea grain (data averaged over two years)

	Fat	Neutral detergent fiber	Acid detergent fiber	Cellulose	Hemi-cellulose	Ash	Organic matter
				%			
Cultivars							
CL 367	1.18	40.1a	11.1	6.95b	28.9a	3.71b	96.3a
Cowpea 88	1.14	38.5b	11.1	7.20a	27.4b	4.20a	95.8b
LSD ($p = 0.05$)	NS	1.16	NS	0.15	1.20	0.06	0.06
Bio-regulators							
Control	1.32a	40.1a	11.5	7.02	28.7a	4.10a	95.9c
Sodium benzoate (100 µg mL^{-1})	1.28ab	40.4a	11.1	7.30	29.4a	3.91b	96.1b
Sodium benzoate (150 µg mL^{-1})	1.24ab	38.4a	10.8	7.06	27.5a	3.90b	96.1b
Salicylic acid (50 µg mL^{-1})	0.95c	35.7b	11.0	7.08	24.7b	3.74c	96.3a
Salicylic acid (100 µg mL^{-1})	1.07bc	40.2a	11.1	6.96	29.1a	4.02ab	96.0bc
$CaCl_2$ (0.5%)	1.17b	40.9a	11.3	7.13	29.6a	4.06ab	95.9c
$CaCl_2$ (1.0%)	1.08bc	39.7a	10.9	6.90	28.8a	3.91b	96.1b
KNO_3 (1.0%)	1.26ab	39.5a	11.2	7.05	28.3a	3.96b	96.0bc
KNO_3 (2.0%)	1.07bc	38.4a	11.2	7.17	27.2b	3.93b	96.2ab
LSD ($p = 0.05$)	0.13	2.50	NS	NS	2.50	0.13	0.10

Table 4: Cowpea grain yield and grain quality parameters in different years (data pooled across the cultivars and foliar treatments)

Year	Biological Yield (q ha^{-1})	Pod yield (q ha^{-1})	Grain yield (q ha^{-1})	N (%)	P (%)	K (%)	Crude protein (%)	CP yield (q ha^{-1})	Fat %	NDF (%)	Cellulose (%)	Hemi-cellulose (%)	Ash (%)	OM (%)	Zn	Cu	Fe	Mn
															(mg kg^{-1})			
2008	105.0a	19.0a	14.0a	4.0a	0.64a	1.47a	25.1a	3.63a	1.2a	40.2a	7.0	29.0a	3.0b	95.9b	39.4a	3.2	36.9a	14.1a
2009	99.0b	17.0b	13.0b	3.8b	0.59b	1.44b	23.6b	3.19b	1.1b	38.3b	7.1	27.3b	3.8a	96.1a	35.0b	3.1	33.0b	12.8b
LSD (p=0.05)	4.0	0.6	0.4	0.1	0.01	0.02	0.48	0.12	0.06	1.2	NS	1.2	0.06	0.06	1.1	NS	1.0	0.4

Means sharing the same letter in a column do not differ significantly at $p = 0.05$; NS= Non significant

Discussion

Cultivars of cowpea recorded different biological, pod and grain yield in our study. Peksen (2007) also recorded varietal differences in seed and biological yield of cowpea. The observed differences among genotypes for various yield parameters could be attributed to the intrinsic differences in the ability of different cultivars to utilize available growth resources (Makoi and Ndakidemi, 2010). Also late flowering in cultivar CL 367 might had provided more time to plants for manufacturing of more photo-assimilates and thus more biological, pod and grain yield than Cowpea 88 cultivar.

Different bio-regulators can manipulate plant responses and modify plant gene expression, affecting levels of DNA, RNA, enzymes and hence has significant effect on yield and quality of crops (Olaiya *et al.,* 2013). Sodium benzoate application in plants inhibits ethylene production and delays senescence resulting in accumulation of more photoassimilates and hence increase the yield of cereal and legume crops under heat stress condition (Beltrano *et al.,* 1999; Kumar *et al.,* 2013). In our study also, foliar application of sodium benzoate led to increase in biological, pod and grain yield, which might be due enhanced assimilation of photo assimilates.

Information available in literature is meager regarding the effect of exogenously applied bio-regulator like salicylic acid on grain yield of forage cowpea. Increase in pod and grain yield of cowpea by using bio-regulator salicylic acid might be due to enhanced assimilation, increased nutrient uptake and photosynthesis, improved assimilates translocation, cytoplasmic streaming and increased cell integrity in plants (Wahid *et al.,* 2007). Exogenous application of salicylic acid to the plants increased total antioxidant capacities, prevents damage to cell membranes (Wahid *et al.,* 2007), inhibit ethylene production under high night temperature (Kumar *et al.,* 2013) and is able to increase crop yields by increasing the uptake of NPK and other minerals from soil. Salicylic acid plays a key role in providing tolerance against temperature stress. Foliar spray of lower concentrations of salicylic acid conferred heat tolerance to mustard and maize due to enhanced H_2O_2 level and reduced the catalase (CAT) activity, thereby increasing the potential of plants to withstand heat stress and avoiding loss in grain yield and its quality (Hayat *et al.,* 2010). Mandavia *et al.* (2010) found that application of 50 and 100 µg mL^{-1} of salicylic acid as foliar spray at vegetative (40 days after sowing) and reproductive stage (55 days after sowing) on chickpea had significantly increased the seed yield as compared to control. A decrease in pod and grain yield under salicylic acid at 100 µg mL^{-1} in our study might be due to inhibition of foliar protein which leads to breakdown/degradation of chlorophyll (Wahid *et al.,* 2007; Kumar *et al.,* 2013).

The increase in pod and grain yield by application of 1% $CaCl_2$ might be due to availability of Ca^{+2} to plants which plays an important role in many biochemical processes, delaying senescence, regulation of plant activities, especially through protein phosphorylation and controlling physiological disorders in fruits and vegetables (Wahid *et al.,* 2007; Rab and Haq, 2012). El-Abou (2011) also reported that foliar application of 500 µg mL^{-1} chelated Ca to *Phaselous vulgaris* at 40 and 60 days after sowing recorded significantly more number of fruit sets and seed yield as compared to control.

An increase in pod and grain yield with the application of KNO_3 might be due to availability of K from KNO_3, which has important role in growth and development of plant as it increases enzyme activity and photosynthesis, improve synthesis of protein, carbohydrates and fats and translocation of photosynthates from source to sink, maintenance of turgor and transpiration in cells under environmental stress conditions. Plants fertilized with N and K had better tolerance to photo-oxidative damage and has higher photosynthetic capacity. The increment of yield under heat stress conditions by foliar application of KNO_3 was also reported in Egyptian clover by Kumar *et al.* (2013).

Little information is available in the literature regarding the effect of foliar application of bio-regulators on the proximate analysis of cowpea grain. Proximate analysis of grain was influenced due to different cultivars and bio-regulators. Pannu *et al.* (2008) also recorded difference in various nutritive values of crude protein, NDF, ADF, cellulose and hemicelluloses for green fodder of cultivar CL 367 and Cowpea 88.

Bio-regulators act at gene level of plant influencing the translational and transcriptional mechanisms and on the transaminase enzyme level in the plant (Karim *et al.,* 2006) for N and protein synthesis. Bio-regulators application on foliage can improve the nutrition of the plant and seed or grain (Wahid *et al.,* 2007). In this regard Mandavia *et al.* (2006); Devi *et al.* (2011) reported that exogenously applied salicylic acid enhanced nitrogen and crude protein content of soybean plant and seed due to enhanced activity of nitrate reductase enzyme. Salicylic acid might be involved in mobilization of internal tissue NO_3^- and chlorophyll biosynthesis to increase the functional state of the photosynthetic machinery in plants (Wahid *et al.,* 2007; Hayat *et al.,* 2010), or may induce accumulation of α-amino levulinic acid (α-ALA) in the cotyledons (Singh *et al.,* 2010). Karim *et al.* (2006) found lowest values of ether extract (7.94%) and crude fiber (4.74%) content of chickpea seed in treatment where foliar application of 20 µg mL^{-1} NAA was applied on the crop at 45 days after sowing.

Higher level of macro- and micro nutrients in grains of Cowpea 88 cultivar may be due to better partitioning of photo-assimilates between less numbers of pods present on the plants. The difference in micronutrient content of grain may also be due to intrinsic ability of genotype to access different growth resources (Makoi and Ndakidemi, 2010). Foliar spray of bio-regulators on plant can enhance nutritive quality of grain due to enhanced translocation of photosynthates in the grains (Devi *et al.,* 2011; Rasheed *et al.,* 2011; Kumar *et al.,* 2013; Olaiya *et al.,* 2013). Also application of bio-regulators encourages the uptake of nitrogen from the soil and may activate transaminase enzyme which in turn increases the quality of grain (Karim *et al.,* 2006). Amin *et al.* (2008) reported that foliar spray of salicylic acid enhanced the N, P, K, Zn, Cu, Na content in wheat grains. Foliar spray of 50 µg mL^{-1} of salicylic acid led to increase in oil and crude protein content of soybean seed due enhanced nutrient uptake from soil had also been reported by Devi *et al.* (2011).

As regards years, yield parameters were significantly higher in the year 2008 than 2009 (Table 4). Grain yield was 7.7% higher during the year 2008 than 2009. NPK content of grain also recorded the same trend. Crude protein content and crude protein yield was also found to be significantly higher during the first year of study. Fat, NDF, hemi-cellulose, micronutrient content of grains were more in the year 2008. However, ash and organic matter content of grain was higher during the year 2009. Higher rainfall and lower night temperature during flowering and reproductive period during the year 2008 might have provided a good micro-environment for the growth of cowpea, which presumably resulted in higher grain yield and its nutritional quality. Babaji *et al.* (2011) also recorded better performance of cowpea in terms of growth parameters, grain yield and quality in the year with higher rainfall and milder temperatures. Decreases in grain yield of cowpea due to excessive floral abscission, lower pod set, reduced photosynthesis, and lower seed weight at high night temperatures (>20°C) had been reported by Mutters and Hall (1992) and Thuzar *et al.* (2010).

In conclusion, cowpea cultivar CL 367 had shown promising results related to biological, pod, grain and crude protein yield than the cultivar Cowpea 88. Among the various bio-regulators, three foliar sprays of salicylic acid at 50 µg mL^{-1} followed by 2% KNO_3 and 1% calcium chloride starting from flower initiation at an weekly interval recorded promising effect on the grain yield and its nutritional quality. Among the bio-regulators, salicylic acid was more economical compared with KNO_3 and $CaCl_2$. The increased availability of seed/grains of cowpea certainly will enhance the area under this crop thereby improving the productivity of livestock in South-East Asia and other tropical countries.

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