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Effect of Leaf Extracts Seed Priming and Foliar Spray of Panchagavya on Chlorophyll Content, Gaseous Exchange Parameters, Nitrogen, Potassium and Protein Content of Seed in Blackgram [*Vigna mungo* (L.) Hepper] cv. CO 6

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Abstract

The experiment was conducted to study about the effect of various leaf extracts seed priming and foliar spray of Panchagavya on chlorophyll content, gaseous exchange parameters, Nitrogen (N), Potassium (K) and Protein (P) content of seed in blackgram. The seeds of blackgram cv. CO 6 were imposed with leaf extracts seed priming and foliar spray *viz., Moringa* leaf extract 1%, *Prosopis* leaf extract 1%, *Neem* leaf extract 1%, *Pungam* leaf extract 1%, *Arappu* leaf extract 1% and foliar spray of *Moringa* leaf extract 3%, *Panchagavya* 3%, *Prosopis* leaf extract 3% and *Neem* leaf extract 3% along with control. The experiment revealed that leaf extract seed priming with *Prosopis* leaf extract 1% and foliar spray of *Panchagavya* 3% records the higher values for chlorophyll content, gaseous exchange parameters, Nitrogen (N), Potassium (K) and Protein (P) content of seed when compared to other treatments and control.

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Keywords

Leaf Extracts, Seed Priming, *Prosopis* leaf extract, Foliar spray, *Panchagavya*, Chlorophyll content.

Introduction

Blackgram is one of the important annual pulse crop and it's native to central Asia. Pulses are a major source of protein supplement on daily diets based on cereals and starchy food for a predominantly vegetarian population. Therefore pulses are often regarded as poor man's meat (Sooraj Chandra Pankaj and Pradeep Kumar Dewangan, 2017). Popularity of blackgram (*Vigna mungo* L.) is mainly because of its superior nutritional quality and can very well fit into multiple cropping systems, because of short duration (Rajeshkumar *et al.,* 2017). In India the area under blackgram cultivation 3.30 million ha producing 1.60 million tons, with the mean productivity of 0.49 kg ha^{-1} and contributes 11% of total production (Choudhary *et al.,* 2017). Blackgram is a leguminous crop, contains deep taproot system that can helps in holding of soil particles which aids in prevention of soil erosion. The low productivity of pulses was mainly due to the low yield potential, non-synchronous maturity, lack of good quality seeds and crop management. To overcome this adverse conditions seed priming treatments and foliar spray were given prior to the plants. Seed priming is a controlled hydration process which involves exposing seeds to low water potentials that restrict germination but permits pre-germinative physiological and biochemical changes to occur (Heydecker and Coolbear, 1977; Bradford, 1986; Khan, 1992). Nowadays, seed priming has been identified as an integrated common seed treatment to reduce the time between seed sowing and seedling emergence and the synchronization of emergence (Parera and Cantliffe, 1990). Priming enhanced seed performance activity which are related to the repair and the buildup of nucleic acid, enhanced synthesis of protein, repair of membranes and improves antioxidant system (McDonald, 1999; Hsu *et al.,* 2003). Foliar spray application is a common practice of supplying nutrients to plants through their foliage. It involves spraying water-dissolved fertilizers directly on the leaves. Foliar fertilization is a simple and effective method of providing nutrients to crops (Alexander and Schroeder, 1987).

Foliar nutrients which facilitate easy and quick absorption of nutrients by penetrating the stomata or leaf cuticle and enters the cells (Rajeshkumar *et al.,* 2017). Foliar feeding targets the growth stages where declining rates of photosynthesis and levelling off of root growth and nutrient absorption occur, in attempts to aid translocation of nutrients into seed or vegetative production. Keeping the points in view an investigation was carried out to study the effect of leaf extracts seed priming and foliar spray of panchagavya on chlorophyll content, gaseous exchange, nitrogen, potassium and protein content of seed in blackgram.

Materials and Methods

The present investigation was carried out at the Experimental Farm, Sevathur Village, Tiruppatthur Taluk, Vellore District, Tamil Nadu, to study the effect of leaf extracts seed priming and foliar spray of panchagavya on chlorophyll content, gaseous exchange parameters, Nitrogen (N), Potassium (K) and Protein (P) content of seed in blackgram. Cleaned and graded seeds were used for this study. The cleaned and graded seeds were pre-conditioned by keeping the seeds in between two layers of moist gunny bag for one hour to avoid soaking injury. Then the pre-conditioned seeds were soaked in respective solutions at 1/3rd volume of seeds for four hours and the seeds were air dried in shade to their original moisture content. For foliar spray 3 litre of solution was mixed with 100 litre of water to get 3% concentration and then apply by the help of hand sprayer (Gunasekar *et al.,* 2018). The blackgram were given with the following seed treatments and foliar spray at different levels.

Treatment details: Seed priming

T_0 – Control
T_1 – *Moringa* leaf extract 1%
T_2 – *Prosopis* leaf extract 1%
T_3 – *Neem* leaf extract 1%
T_4 – *Pungam* leaf extract 1%
T_5 – *Arappu* leaf extract 1%

Treatment details: Foliar spray

T_0 – Control
T_1 – *Moringa* leaf extract 3%
T_2 – *Panchagavya* 3%
T_3 – *Prosopis* leaf extract 3%
T_4 – *Neem* leaf extract 3%

The experiment was laid out in Randomized Block Design (RBD) with four replications. The plot size was 4 × 2.5 m^2. The crop was raised with the spacing of 30 × 10 cm and recommended package of practices for blackgram were followed. The chlorophyll content was calculated by using the formula which was suggested by Yoshida *et al.,* (1971) and expressed in mg g^{-1}.

The gaseous exchange parameters were measured by using LICOR-6400 × T portable photosynthetic system (Lioncoln, USA). The Nitrogen (N), Potassium (K) and Protein content of seed was estimated by using some procedures which was suggested by Humphries (1956); Piper (1966); Ali-khan and Youngs (1973). The above observations were measured, calculated and statistically analyzed by using ANOVA.

Results and Discussion

Leaf extracts seed priming

Chlorophyll a (mg g^{-1})

Among the seed treatments, *Prosopis* leaf extract 1% primed seed (T_2) recorded the highest chlorophyll a content (1.02) and was followed by primed seed with *Moringa* leaf extract 1% (T_1) (0.95) and the untreated seed (T_0) recorded the lowest number of chlorophyll content a (0.50).

Chlorophyll b (mg g^{-1})

Among the seed treatments, *Prosopis* leaf extract 1% primed seed (T_2) recorded the highest chlorophyll b content (1.10) and was followed by primed seed with *Moringa* leaf extract 1% (T_1) (0.95) and the untreated seed (T_0) recorded the lowest number of chlorophyll content b (0.67).

Total chlorophyll (mg g^{-1})

Significant results were obtained among the seed treatments, *Prosopis* leaf extract 1% primed seed (T_2) recorded the highest total chlorophyll content (2.12) and was followed by primed seed with *Moringa* leaf extract 1% (T_1) (1.90) and the untreated seed (T_0) recorded the lowest number of total chlorophyll (1.17).

The increased chlorophyll 'a' content by 20.00%, chlorophyll 'b' content by 69.23% and total chlorophyll content by 41.33% was recorded by T_2 over the control (T_0). This increase in chlorophyll content may be due to the maximum production of chemical energy and plant metabolism which results in plant growth (Ayumi *et al.,* 2004). This increase could be also due to the presence of mineral nutrients like nitrogen, potassium and calcium which plays a major role in chlorophyll synthesis.

Gas exchange parameters

Among the gas exchange parameters studied *viz.,* photosynthetic rate, transpiration rate, intercellular CO_2 concentration and stomatal conductance, it was found that plants treated with *Prosopis* leaf extract 1% primed seed (T_2) recorded more photosynthetic and transpiration rates (27.00 mg CO_2 $m^{-2}s^{-1}$ and 9.75 mg H_2O $m^{-2}s^{-1}$) followed by primed seed with *Moringa* leaf extract 1% (T_1) (25.75 mg CO_2 $m^{-2}s^{-1}$ and 9.00 mg H_2O $m^{-2}s^{-1}$) and the untreated seed (T_0) recorded the less photosynthetic and transpiration rates (20.50 mg CO_2 $m^{-2}s^{-1}$ and 6.00 mg H_2O $m^{-2}s^{-1}$). For the intercellular CO_2 concentration and stomatal conductance, higher values was recorded by plants treated with *Prosopis* leaf extract 1% primed seed (T_2) (276.25µ mol mol^{-1} and 0.85 mol $m^{-2}s^{-1}$) followed by primed seed with *Moringa* leaf extract 1% (T_1) (245.00µ mol mol^{-1} and 0.82 mol $m^{-2}s^{-1}$) and the untreated seed (T_0) recorded the lower values (200.00µ mol mol^{-1} and 0.60 mol $m^{-2}s^{-1}$).

Among the gas exchange parameters studied *viz.,* 31.71% increase in photosynthetic rate, 62.50% increase in transpiration rate, 38.13% increase in intercellular CO_2 concentration and 41.67% increase in stomatal conductance was recorded by T_2 over the control (T_0).

Increased gas exchange parameters may be due to increased enzyme activity, presence of growth promoting substances like GA_3 and also required essential nutrients that could have sufficiently influence the physiology of leaf as evident by increase in leaf area and chlorophyll content that leads more production of photo-assimilates, better growth and development. Similar results of increased gas exchange parameters *viz.,* leaf photosynthetic rate, transpiration, stomatal conductance and intercellular CO_2 concentration was recorded by Anbarasan (2011) in cowpea and Prakash *et al.,* (2013) in rice.

Nitrogen content of seed (%)

The *Prosopis* leaf extract 1% primed seed (T_2) recorded the highest nitrogen content (1.68%) and was followed by *Moringa* leaf extract 1% primed seed (T_1) (1.67%) and the untreated seed (T_0) recorded the lowest nitrogen content (1.43%).

Potassium content of seed (%)

The *Prosopis* leaf extract 1% primed seed (T_2) recorded the highest potassium content (1.59%) and was followed by *Moringa* leaf extract 1% primed seed (T_1) (1.57%) and the untreated seed (T_0) recorded the lowest potassium content (1.35%).

Protein content of seed (%)

The *Prosopis* leaf extract 1% primed seed (T_2) recorded the highest protein content (22.88%) and was followed by *Moringa* leaf extract 1% primed seed (T_1) (22.82%) and the untreated seed (T_0) recorded the lowest protein content (22.66%).

Almost 14.88% increase in nitrogen content, 15.09% increase in potassium content and 0.96% increase in protein content was recorded by *Prosopis* leaf extract 1% primed seed (T_2) over the control (T_0). The increase in nitrogen and potassium may be due to the nutrients present in leaf extract helped in observation of more nutrients and translocation of nutrient from source to sink. Similar findings were made by Renugadevi and Vijayageetha (2006); Reka (2013). Increase in protein content may be due to the presence of calcium in *Prosopis* leaf extract which acts as an enzyme cofactor in stimulating and accumulation of protein synthesis (Christansen and Foy, 1979).

Foliar spray

Chlorophyll a (mg g^{-1})

Among the foliar spray treatments, *Panchagavya* 3% (T_2) recorded the highest chlorophyll a content (1.02) and was followed by *Moringa* leaf extract 3% (T_1) (0.95) and the untreated plant (T_0) recorded the lowest number of chlorophyll content a (0.63).

Chlorophyll b (mg g^{-1})

Among the foliar spray treatments, *Panchagavya* 3% (T_2) recorded the highest chlorophyll b content (1.10) and was followed by *Moringa* leaf extract 3% (T_1) (0.95) and the untreated plant (T_0) recorded the lowest number of chlorophyll content b (0.67).

Total chlorophyll (mg g^{-1})

Significant results were obtained among the foliar spray treatments, *Panchagavya* 3% (T_2) recorded the highest total chlorophyll content (2.12) and was followed by *Moringa* leaf extract 3% (T_1) (1.90) and the untreated plant (T_0) recorded the lowest number of chlorophyll (1.30).

Significant increase in chlorophyll 'a', 'b' and total chlorophyll content 61.90%, 64.18% and 63.08% respectively may be associated with the supply of essential nutrients to the plants. Since chlorophyll synthesis in the plants is directly related to the availability of the physiologically active Fe, N, P and S micronutrients in available form. Hence these nutrients help the plant in the formation of chlorophyll in the leaves. Increased chlorophyll 'a', 'b' and carotenoids content in green leaves with foliar application of organic solution has also been observed by Tejada and Gonzalez (2003); Subramanian (2005) and Tharmaraj *et al.,* (2011).

Gas exchange parameters

Among the gas exchange parameters studied *viz.,* photosynthetic rate, transpiration rate, intercellular CO_2 concentration and stomatal conductance, it was found that plants foliar sprayed with *Panchagavya* 3% (T_2) recorded more photosynthetic and transpiration rates (27.00 mg CO_2 $m^{-2}s^{-1}$ and 9.50 mg H_2O $m^{-2}s^{-1}$) followed by *Moringa* leaf extract 3% (T_1) (26.50 mg CO_2 $m^{-2}s^{-1}$ and 9.00 mg H_2O $m^{-2}s^{-1}$) and the untreated plant (T_0) recorded less photosynthetic and transpiration rates (20.00 mg CO_2 $m^{-2}s^{-1}$ and 6.00 mg H_2O $m^{-2}s^{-1}$).

For the intercellular CO_2 concentration and stomatal conductance, higher values recorded by plants foliar sprayed with *Panchagavya* 3% (T_2) (275.25µ mol mol^{-1} and 0.85 mol $m^{-2}s^{-1}$) followed by *Moringa* leaf extract 3% (T_1) (265.35µ mol mol^{-1} and 0.78 mol $m^{-2}s^{-1}$) and the untreated plant (T_0) recorded the lower values (225.00µ mol mol^{-1} and 0.65 mol $m^{-2}s^{-1}$). Among the gas exchange parameters studied *viz.,* increase in photosynthetic rate by 35.00%, transpiration rate by 58.33%, intercellular CO_2 concentration by 22.33% and stomatal conductance by 30.77% was recorded by T_2 and lower values recorded by (T_0). This increase may be due to the efficient utilization of nutrients, elimination of losses through leaching and fixation helps in regulating the uptake of nutrient by plants Manonmani and Srimathi (2009). Increase in number of leaves with leaf area could be constructed as an indication of enhanced photosynthetic efficiency in T_2 (Somasundaram *et al.,* 2007).

Increase in gas exchange parameters recorded by T_2 may be due to the presence of effective microorganism in *Panchagavya* stimulated synthesize of phyto hormones (i.e.) auxins (IAA), gibberellin, cytokinin and other plant nutrients which stimulates plant growth and they also contain proactive substances (microbial metabolites) that could significantly affect leaf stomatal response according to the water status of the plant (Xu, 2001; Somasundaram and Amanullah, 2007) which helps in maintaining the opening of stomata for longer period both in optimum and adverse condition during the crop growth which leads to increased LAI providing stronger source for sink (Xu *et al.,* 2000).

Nitrogen content of seed (%)

Maximum nitrogen values was recorded by *Panchagavya* 3% (T_2) foliar sprayed plant (1.65%) and was followed by *Moringa* leaf extract 3% (T_1) (1.60%) and seeds from the untreated plant (T_0) recorded the lowest nitrogen content (1.45%).

Potassium content of seed (%)

The *Panchagavya* 3% (T_2) foliar sprayed plant recorded the highest potassium content (1.53%) and was followed by *Moringa* leaf extract 3% (T_1) (1.52%) and seeds from the untreated plant (T_0) recorded the lowest potassium content (1.34%).

Protein content of seed (%)

The *Panchagavya* 3% (T_2) foliar sprayed plant recorded the highest protein content (22.89%) and was followed by *Moringa* leaf extract 3% (T_1) (22.84%) and seeds from the untreated plant (T_0) recorded the lowest protein content (22.68%).

Table.1 Effect of leaf extracts seed priming on chlorophyll content in blackgram cv. CO 6

Treatment (T)	Chlorophyll (mg g^{-1})		
	a	b	Total
T_0	0.50	0.67	1.17
T_1	0.95	0.95	1.90
T_2	1.02	1.10	2.12
T_3	0.85	0.65	1.50
T_4	0.75	0.75	1.50
T_5	0.60	0.72	1.32
Mean	0.77	0.80	1.58
SEd	0.0132	0.0088	0.0627
CD (P=05)	0.0294	0.0196	0.1399

Table.2 Effect of leaf extracts seed priming on gaseous exchange parameters in blackgram cv. CO 6

Treatment (T)	Pn (mg CO_2 $m^{-2}s^{-1}$)	Tr (mg H_2O $m^{-2}s^{-1}$)	Ci CO_2 Concentration (μ mol mol^{-1})	CS (mol $m^{-2}s^{-1}$)
T_0	20.50	6.00	200.00	0.60
T_1	25.75	9.00	245.00	0.82
T_2	27.00	9.75	276.25	0.85
T_3	24.75	8.00	248.75	0.77
T_4	26.50	8.25	253.75	0.80
T_5	24.00	7.00	243.75	0.75
Mean	24.75	8.00	244.58	0.76
SEd	0.5171	0.0671	1.9736	0.0088
CD (P=05)	1.1532	0.1497	4.4012	0.0196

Table.3 Effect of leaf extracts seed priming on nitrogen content (%), potassium content (%) and protein content (%) of seed in blackgram cv. CO 6

Treatment (T)	Nitrogen content of seed (%)	Potassium content of seed (%)	Protein content of seed (%)
T_0	1.43 (6.80)	1.35 (6.71)	22.66 (28.42)
T_1	1.67 (7.49)	1.57 (7.27)	22.82 (28.52)
T_2	1.68 (7.49)	1.59 (7.27)	22.88 (28.59)
T_3	1.62 (7.27)	1.54 (7.04)	22.73 (28.45)
T_4	1.63 (7.27)	1.56 (7.27)	22.77 (28.52)
T_5	1.57 (7.27)	1.48 (7.04)	22.69 (28.45)
Mean	1.60 (7.26)	1.51 (7.10)	22.75 (28.49)
SEd	0.0022 (0.0002)	0.0047 (0.0481)	0.0091 (0.0135)
CD (P=05)	0.0047 (0.0005)	0.0102 (0.1049)	0.0199 (0.0294)

(Figures in parenthesis indicate arcsine transformed value)

Table.4 Effect of foliar spray of panchagavya on chlorophyll content in blackgram cv. CO 6

Treatment (T)	Chlorophyll (mg g^{-1})		
	a	b	Total
T_0	0.63	0.67	1.30
T_1	0.95	0.95	1.90
T_2	1.02	1.10	2.12
T_3	0.71	0.75	1.46
T_4	0.83	0.76	1.59
Mean	0.82	0.84	1.67
SEd	0.0167	0.0017	0.0449
CD (P=05)	0.0387	0.0040	0.1038

Table.5 Effect of foliar spray of panchagavya on gaseous exchange parameters in blackgram cv. CO 6

Treatment (T)	Pn (mg CO_2 $m^{-2}s^{-1}$)	Tr (mg H_2O $m^{-2}s^{-1}$)	Ci CO_2 Concentration (μ mol mol^{-1})	CS (mol $m^{-2}s^{-1}$)
T_0	20.00	6.00	225.00	0.65
T_1	26.50	9.00	265.35	0.78
T_2	27.00	9.50	275.25	0.85
T_3	25.55	8.05	243.75	0.75
T_4	26.00	8.20	245.00	0.77
Mean	25.01	8.15	250.87	0.76
SEd	0.1760	0.0491	0.4994	0.0045
CD (P=05)	0.4066	0.1135	1.1536	0.0103

Table.6 Effect of foliar spray of panchagavya on nitrogen content (%), potassium content (%) and protein content (%) of seed in blackgram cv. CO 6

Treatment (T)	Nitrogen content of seed (%)	Potassium content of seed (%)	Protein content of seed (%)
T_0	1.45 (6.96)	1.34 (6.63)	22.68 (28.45)
T_1	1.60 (7.27)	1.52 (7.11)	22.84 (28.54)
T_2	1.65 (7.41)	1.53 (7.04)	22.89 (28.59)
T_3	1.50 (7.04)	1.48 (7.04)	22.72 (28.45)
T_4	1.53 (7.04)	1.49 (7.04)	22.79 (28.52)
Mean	1.54 (7.14)	1.47 (6.97)	22.78 (28.51)
SEd	0.0069 (0.0686)	0.0225 (0.0716)	0.0111 (0.0148)
CD (P=05)	0.0155 (0.1531)	0.0501 (0.1597)	0.0248 (0.0329)

(Figures in parenthesis indicate arcsine transformed value)

Increased levels of nitrogen content (12.12%), potassium content (12.41%) and protein content (0.91%) was recorded by T_2 over the control (T_0). The increase in nitrogen and potassium content is due to the mode of action of *Panchagavya* and has the ability to bring the flow of cosmic energy. The cosmic energy when made to pass through a living system removes the imbalance in terms of physical, chemical, biological aspects and harmonizes the basic elements which revitalize the crop growth process (Somasundaram, 2003). Increase in protein content was due to the enzymatic activity of nitrate reductase and glutamate synthase present in *Panchagavya* as reported by Vijayakumari *et al.,* (2012).

Conclusion

Hence, the present study concludes that the effect of leaf extracts seed priming and foliar spray of Panchagavya on chlorophyll content, gaseous exchange parameters, Nitrogen (N), Potassium (K) and Protein (P) content of seed in blackgram records the increased values by treating the seeds with *Prosopis* leaf extract 1% and foliar spray with *Panchagavya* 3%. This increase was due to the presence of chemical energy and plant metabolism, increased enzyme activity, nutrients present in *Prosopis* leaf extract, elimination of losses through leaching and fixation helps in regulating the uptake of nutrient and presence of effective microorganism in *Panchagavya.*

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