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Studies on Influence of Lipo-chito Oligosaccharides, Nano and Water-Soluble Fertilizer on Growth and Growth Parameters of Soybean [*Glycine max* (L.) Merrill]

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A field experiment was conducted at University of Agricultural Sciences, GKVK, Bengaluru during *kharif* 2022 to study the influence of lipo-chito oligosaccharides, nano and water-soluble fertilizer on growth and yield of soybean [*Glycine max* (L.) Merrill]. The experiment was laid out in randomized

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complete block design with ten treatments and replicated thrice. The results revealed that application of 75% recommended dose of NP + 100 % K along with soil application of lipo-chito oligosaccharide (LCO) fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of 19:19:19 @ 2 % spray at flowering and pod filling stages recorded significantly higher leaf area, leaf area index at 60 DAS (1772 cm^2 $plant^{-1}$ and 4.91 respectively), leaf area duration (57.59 and 81.07 cm^2 day^{-1} at 30-60 DAS and 60 DAS-harvest, respectively), no. of nodules plant at 35 DAS (31.12), total dry matter accumulation (12.74 and 20.05 g $plant^{-1}$ at 60 DAS and harvest, respectively), absolute growth rate (0.24 and 0.30 g day^{-1} at 30-60 DAS and 60 DAS-harvest, respectively), crop growth rate (8.15 and 9.21 g cm^{-2} day^{-1} at 30-60 DAS and 60 DAS-harvest, respectively) and relative growth rate (0.031 and 0.040 g g day^{-1} at 30-60 DAS and 60 DAS-at harvest, respectively).

Keywords: Soybean; LCO, WSF; nano DAP.

1. INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] belongs to the family *Fabaceae*. Soybean [*Glycine max* (L.) Merrill] is also known as the "wonder crop" of the 20th century and also the most affordable source of both protein and vegetable oil. Because of its significant economic impact and great nutritional content, soybean is known as the “golden bean”. It has a protein content of roughly 40 per cent with well-balanced essential amino acids, 20 per cent oil rich in polyunsaturated fatty acids, 7 per cent mineral content, 6 per cent crude fiber and 17–19 per cent carbohydrates. Soybean protein quality is on par with that of meat, milk, and eggs. It is farmed in India across an area of 12.14 million hectares, producing 12.98 million tonnes with the productivity of 1069 kilograms per hectare [1]. Madhya Pradesh, Maharashtra, Rajasthan, Karnataka, Andhra Pradesh and Gujarat are the major soybean-producing states in India.

The efficacy of nutrients provided by soil application is frequently reduced as a result of leaching and volatilization losses. Foliar application of nutrients at crucial phases will immediately reach the site of food synthesis, resulting in no waste and a rapid supply of photosynthates while lowering the need for fertilizers. In comparison to other fertilization techniques, foliar spray helps in quicker absorption and utilization of nutrients. It is also known that active nodulation of soybean ceases at 45–50 days after planting and at this point, providing nutrients to legume plants by foliar spray has been shown to have positive impacts on promoting growth, boosting seed output and improving quality metrics. Specific to soybean, leaf withering begins far before pod maturity is complete, breaking the source-sink interaction and ultimately resulting in empty pods and pods with shriveled seeds. These problems can be resolved by applying nutrients topically to the plant.

Lipo-chito oligosaccharides (LCOs) are signaling molecules produced by rhizobial bacteria, which start the nodulation process in legumes and by certain fungi, particularly the arbuscular and ecto-mycorrhizal fungi, which also form symbiotic associations with plants. LCOs made by *Brady Rhizobium japonicum* are pentameric molecules containing 2-0-methyl fructose at the reducing end of the chitin backbone and C18:1, C16:1, and C16:0 fatty acid chains at the non-reducing end [2]. Water soluble fertilizers are utilized as chemical fertilizers for foliar spray to supplement crop growth and quality. To minimize the risk of burning plant tissue, water-soluble fertilizers are 100% soluble in water with a low salt index, making them appropriate for foliar application. Nano fertilizers have a gradual release mechanism that prolongs nutrient availability, decreases losses and synchronizes nutrient release with crop development, all of which contributes to a higher nutritional efficiency. By combining all these, the current study "Studies on influence of lipo-chito oligosaccharides, nano and water-soluble fertilizer on growth and yield of soybean (*Glycine max* L.)" has been formulated.

2. MATERIALS AND METHODS

A field experiment was conducted during *kharif*, 2022 at University of Agricultural Sciences, GKVK, Bengaluru on red sandy loam soil having pH 5.63 and EC 0.28 dS m^{-1}. The soil was low in organic carbon content (0.33 %) and available N (273 kg ha^{-1}), and medium in available P_2O_5 (32 kg ha^{-1}) with high available K_2O content (184 kg ha^{-1}). The experimental site was located 13˚ 08’ North latitude and 77˚ 57’ East longitudes with an

altitude of 924 meters above the mean sea level. It comes under the Eastern Dry Zone, of agro-climatic zone "V" of Karnataka. The experiment was laid out in a complete randomized block design with ten treatments replicated thrice. Nutrients were applied as per the recommended package of practices of University of Agricultural Sciences, Bengaluru 25:62.5:25 kg of N: P_2O_5: K_2O ha^{-1} along with different combinations of water soluble NPK (19:19:19), Nano DAP and lipo-chito oligosaccharides as per the treatments and the quantity of spray solution used was 500 litres per hectare. Land preparation was done by ploughing the land with tractor drawn disc plough once and later tractor drawn cultivator was passed twice to remove weeds and to crush the clods, followed by harrowing to bring the soil to a fine tilth. At the time of sowing, the land was prepared to a fine seedbed and the plots were laid out. The treatments consists of control and different combinations of lipo-chito oligosaccharides (LCO) fortified bio-fertilizer, nano and 19:19:19 WSF along with different rates (100 and 75%) of recommended dose of fertilizers. The variety JS-335 was used and fertilizers were applied according to the treatments. The crop was sown on 16th August 2022 with a spacing of 30 × 10 cm. Harvesting was done on 5th November 2022. Five randomly selected plants from each net plot area were harvested separately for recording necessary biometric and yield observations. Border lines were harvested first and removed from the experimental area. Leaf area duration is the integration of leaf area index over a growth period (Watson, 1952). Leaf area duration (LAD) for various growth periods were worked out from the following formula (Power *et al*., 1967) and expressed in days.

Where, L^1 is LAI at time t_1 and L_2 is LAI at time and $t_2 - t_1$ = Time interval between crop growth period in days.

Subsequently, the pods from the net plot area were harvested and allowed for sun-drying for about 4-5 days. After five days of sun-drying, threshing was done manually by beating the pods with a stick there after the seeds were cleaned manually. Treatment wise seed and haulm yield were recorded separately from each net plot after completion of threshing.

The data collected from the experiment at different growth stages and at harvest were subjected to statistical analysis as described by Gomez and Gomez [3]. The level of significance used for 'F' and 't' tests was P=0.05. Critical Difference (CD) values were calculated at 5 per cent probability level.

3. RESULTS AND DISCUSSION

3.1 Effect of Lipo-chito Oligosaccharides (LCOs), Nano and Water-soluble Fertilizer on Growth Parameters of Soybean

3.1.1 Leaf area

The results shown that application of T_{10} treatment i.e., 75 % recommended dose of NP +100 % K along with soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of 19:19:19 @ 2% spray at flowering and pod filling stages has recorded significantly higher leaf area per plant at 60 DAS (1772 cm^2) and it was on par with treatment (T_6) *i.e.,* 100 % RDF along with foliar application of WSF 19:19:19 @ 2% spray at flowering and pod filling stages (1745 cm^2). This higher leaf area might be due to This higher leaf area might be due to nitrogen, which was improved due to the application of LCOs. In particular, N plays a significant role in leaf growth and greenness. It is a crucial component of chlorophyll, the pigment responsible for photosynthesis. The synergistic effect of macro nutrient which was applied through water soluble fertilizer helped in improvement of photosynthesis and various biochemical processes which responds towards growth [4].

3.1.2 Leaf area index

Significantly, higher leaf area index (4.91) was recorded in treatment with application of 75% recommended dose of NP and 100 % K along with soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of 19:19:19 @ 2% spray at flowering and pod filling stages at 60 DAS and it was on par with treatment T_6 *i.e*., 100% NPK along with foliar application of WSF 19:19:19 @ 2% spray at flowering and pod filling stages (4.82) and T_8 treatment i.e., 75 % RDF along with foliar application of nano DAP @ 0. 2 % spray at flowering and pod filling stages (4.42). The increase in leaf area index was due to the increased number of leaves and leaf area $plant^{-1}$.

3.1.3 Leaf area duration

Application of 75 % recommended dose of NP +100 % K along with soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of 19:19:19 @ 2% spray at flowering and pod filling stages has recorded significantly

higher leaf area duration at 30 to 60 DAS and 60 DAS to at harvest (57.59 and 81.07 cm^2 day^{-1}) and it was on par with the treatment T_6 *i.e.*, 100 % RDF along with foliar application of WSF 19:19:19 @ 2% spray at flowering and pod filling stages (56.39 and 79.53 cm^2 day^{-1}) and T_8 treatment i.e., 75 % RDF along with foliar application of nano DAP @ 0. 2 % spray at flowering and pod filling stages (54.53 and 77.21 cm^2 day^{-1}). This might be due to adequate supply of macro nutrients NPK obtained from foliar spray of WSF and also improved biological nitrogen fixation which promoted leaf growth and greenness. Nitrogen is a key component of chlorophyll, which is essential for photosynthesis. Adequate nitrogen supports the development of new leaves and helps maintain the existing ones.

3.1.4 Number of nodules per plant

Application of 75% recommended dose of NP +100 % K along with soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of 19:19:19 @ 2% spray at flowering and pod filling stages has recorded significantly higher number of nodules per plant at 35 DAS (31.12) and it was on par with treatment T_8 *i.e.,* 75% recommended dose of NP +100 % K along with soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of nano DAP @ 0.2% spray at flowering and pod filling stages (29.67). The increased plant growth might be due to ability of LCOs to have "hormone-like" effects that trigger the "Nod factors". Nod factors are potent inducers of cell division through the induction of cell cycle genes in plants. These nod factors might have induced the new organogenesis of nodules in soybean. Similarly, induction of cell division by "Nod factors" in *Picea abies* has been reported by Dyachock *et al*. [5].

3.1.5 Total dry matter accumulation per plant

The results shown that application of 75 % recommended dose of NP +100 % K along with soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of 19:19:19 @ 2% spray at flowering and pod filling stages has recorded significantly higher total dry matter accumulation per plant at 60 DAS and at harvest (12.74 and 20.05 g $plant^{-1}$ respectively) and it was on par with treatment T_6 *i.e.,* 100 % RDF along with foliar application of WSF 19:19:19 @ 2% spray at flowering and pod filling stages (12.28 and 19.08 g $plant^{-1}$, respectively). Huge increment in plant dry matter production at various phases of development in LCO treated plants might be attributed to enhanced nodulation in soybean, improved root and shoot development and increased nitrogen fixation rate in soybean. In addition, increment in the macro nutrient availability and their impact on various physiological functions such as better assimilation of photosynthates, higher content of chlorophyll and formation of the other nitrogen compounds like amino acids, proteins and protoplasm. Potassium foliar nutrition helped in osmotic regulation and increased metabolic activity of plants, which resulted in increase of plant height, number of leaves and number of branches per plant, thus higher dry matter accumulation per plant [6].

3.2 Absolute Growth Rate

The results shown that application of 75 % recommended dose of NP +100 % K along with soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of 19:19:19 @ 2% spray at flowering and pod filling stages has recorded significantly higher absolute growth rate during 30 to 60 DAS and 60 DAS to at harvest (0.29 and 0.30 g day^{-1}, respectively) followed by treatment T_6 *i.e.,* 100 % RDF along with foliar application of WSF 19:19:19 @ 2% spray at flowering and pod filling stages (0.28 and 0.29 g day^{-1}, respectively). Higher AGR values represents the enhanced vegetative growth and reduced leaf senescence due to application of macro nutrients through foliar spray at flowering and pod filling stages.

3.3 Crop Growth Rate

The results shown that application of 75 % recommended dose of NP +100 % K along with soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of 19:19:19 @ 2% spray at flowering and pod filling stages has recorded significantly higher crop growth rate during 30 to 60 DAS and 60 DAS to at harvest (9.93 and 9.21 g cm^{-2} day^{-1}, respectively) and next higher CGR was recorded in the treatment T_6 i.e., 100% NPK along with foliar application of WSF 19:19:19 @ 2% spray at flowering and pod filling stages (9.54 and 8.64 g cm^{-2} day^{-1}, respectively). Crop growth rate is the rate of increase of plant dry weight per unit ground area per unit time and it is an interaction of NAR and LAI. The response of soybean to LCO showed a significant increase in CGR. Higher the CGR normally likely to enhance the total dry matter production and yield of the crop [7].

Table 1. Leaf area per plant of soybean and number of nodules per plant at different growth stages as influenced by lipo-chito oligosaccharides, nano and water soluble fertilizer

Treatments	Leaf area (cm^2) $plant^{-1}$		No. of nodules plant at 35 DAS
	30 DAS	60 DAS	
T_1 - Absolute control	233	1278	20.33
T_2 - Nutrient management as per PoP (25:62.5:25 kg N, P_2O_5, K_2O)	272	1420	22.50
T_3 - Foliar application of Nano DAP @ 0.2% spray at flowering	277	1454	23.77
T_4 - Foliar application of Nano DAP @ 0.2% spray at flowering and pod filling	274	1632	25.70
T_5 - Foliar application of WSF 19:19:19 @ 2% spray at flowering	273	1497	24.90
T_6 - Foliar application of WSF 19:19:19 @ 2% spray at flowering and pod filling	278	1745	27.92
T_7 - Soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of Nano DAP @ 0.2% spray at flowering	251	1537	26.57
T_8 - Soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of Nano DAP @ 0.2% spray at flowering and pod filling	253	1626	29.67
T_9 - Soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of19:19:19 @ 2% spray at flowering	251	1556	27.11
T_{10} - Soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of 19:19:19 @ 2% spray at flowering and pod filling	259	1772	31.12
S. Em ±	11.50	66.20	1.02
CD at 5 %	NS	196.73	3.04

Note: LCO - Lipo-chito oligosaccharides, WSF - Water soluble fertilizers

- *75% Recommended dose of NP is applied to the soil from T_7 to T_{10} treatments; 100% RD of NP from T_2-T_6 treatments.*
- *100% Recommended dose of K is applied to the soil as basal application from T_2 to T_{10} treatments.*

Table 2. Leaf area index, leaf area duration of soybean at different growth stages as influenced by lipo-chito oligosaccharides, nano and water soluble fertilizer

Treatments	Leaf area index		LAD (cm^2 day^{-1})	
	30 DAS	60 DAS	30 DAS to 60 DAS	60 DAS to at harvest
T_1 - Absolute control	0.78	3.26	39.67	58.31
T_2 - Nutrient management as per PoP (25:62.5:25 kg N, P_2O_5, K_2O)	0.81	3.73	48.59	61.94
T_3 - Foliar application of Nano DAP @ 0.2% spray at flowering	0.92	3.85	51.55	66.74
T_4 - Foliar application of Nano DAP @ 0.2% spray at flowering and pod filling	0.91	4.44	53.12	71.73
T_5 - Foliar application of WSF 19:19:19 @ 2% spray at flowering	0.86	3.99	48.91	72.88
T_6 - Foliar application of WSF 19:19:19 @ 2% spray at flowering and pod filling	0.91	4.82	56.39	79.53
T_7 - Soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of Nano DAP @ 0.2% spray at flowering	0.84	4.12	51.42	73.33
T_8 - Soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of Nano DAP @ 0.2% spray at flowering and pod filling	0.85	4.42	54.53	77.21
T_9 - Soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of 19:19:19 @ 2% spray at flowering	0.84	4.19	52.53	72.73
T_{10} - Soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of 19:19:19 @ 2% spray at flowering and pod filling	0.87	4.91	57.59	81.07
S. Em ±	0.03	0.22	0.97	1.52
CD at 5 %	NS	0.65	2.90	4.54

Note: LCO - Lipo-chito oligosaccharides, WSF - Water soluble fertilizers

- *75% Recommended dose of NP is applied to the soil from T_7 to T_{10} treatments; 100% RD of NP from T_2-T_6 treatments.*
- *100% Recommended dose of K is applied to the soil as basal application from T_2 to T_{10} treatments.*

Table 3. Total dry weight per plant and absolute growth rate of soybean at different growth stages as influenced by lipo-chito oligosaccharides, nano andwater-soluble fertilizer

Treatment details	Total dry weight plant^{-1} (g)		Absolute growth rate (g day^{-1})	
	60 DAS	at harvest	30 DAS to 60 DAS	60 DAS to at harvest
T_1 - Absolute control	6.27	15.83	0.110	0.220
T_2 - Nutrient management as per PoP (25:62.5:25 kg N, P_2O_5, K_2O)	8.57	16.73	0.160	0.250
T_3 - Foliar application of Nano DAP @ 0.2% spray at flowering	9.54	16.87	0.200	0.270
T_4 - Foliar application of Nano DAP @ 0.2% spray at flowering and pod filling	11.34	18.15	0.250	0.280
T_5 - Foliar application of WSF 19:19:19 @ 2% spray at flowering	9.11	17.37	0.190	0.260
T_6 - Foliar application of WSF 19:19:19 @ 2% spray at flowering and pod filling	12.28	19.08	0.280	0.290
T_7 - Soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of Nano DAP @ 0.2% spray at flowering	10.19	17.71	0.230	0.250
T_8 - Soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of Nano DAP @ 0.2% spray at flowering and pod filling	11.35	18.37	0.250	0.270
T_9 - Soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of19:19:19 @ 2% spray at flowering	10.53	17.87	0.220	0.240
T_{10} - Soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of 19:19:19 @ 2% spray at flowering and pod filling	12.74	20.05	0.290	0.300
S. Em±	0.49	0.51	0.004	0.004
CD at 5%	1.48	1.52	0.001	0.001

Note: LCO - Lipo-chito oligosaccharides, WSF - Water soluble fertilizers

- *75% Recommended dose of NP is applied to the soil from T_7 to T_{10} treatments; 100% RD of NP from T_2-T_6 treatments.*
- *100% Recommended dose of K is applied to the soil as basal application from T_2 to T_{10} treatments.*

Table 4. Crop growth rate and relative growth rate of soybean at different growth stages as influenced by lipo-chito oligosaccharides, nano and water-soluble fertilizer

Treatment details	Crop growth rate (g cm^{-2} day^{-1})		Relative growth rate (g g^{-1} day^{-1})	
	30 DAS to 60 DAS	60 DAS to at harvest	30 DAS to 60 DAS	60 DAS to at harvest
T_1 - Absolute control	3.84	6.59	0.0260	0.0190
T_2 - Nutrient management as per PoP (25:62.5:25 kg N, P_2O_5, K_2O)	5.25	7.54	0.0270	0.0230
T_3 - Foliar application of Nano DAP @ 0.2% spray at flowering	6.45	7.70	0.0320	0.0240
T_4 - Foliar application of Nano DAP @ 0.2% spray at flowering and pod filling	8.31	8.61	0.0360	0.0260
T_5 - Foliar application of WSF 19:19:19 @ 2% spray at flowering	5.75	7.66	0.0280	0.0250
T_6 - Foliar application of WSF 19:19:19 @ 2% spray at flowering and pod filling	9.54	8.64	0.0390	0.0280
T_7 - Soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of Nano DAP @ 0.2% spray at flowering	7.75	7.74	0.0370	0.0250
T_8 - Soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of Nano DAP @ 0.2% spray at flowering and pod filling	7.83	8.46	0.0380	0.0270
T_9 - Soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of19:19:19 @ 2% spray at flowering	7.49	8.32	0.0340	0.0260
T_{10} - Soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of 19:19:19 @ 2% spray at flowering and pod filling	9.93	9.21	0.0400	0.0310
S. Em ±	0.10	0.15	0.0006	0.0004
CD at 5%	0.32	0.45	0.0019	0.0014

Note: LCO - Lipo-chito oligosaccharides, WSF - Water soluble fertilizers

- *75% Recommended dose of NP is applied to the soil from T_7 to T_{10} treatments; 100% RD of NP from T_2-T_6 treatments.*
- *100% Recommended dose of K is applied to the soil as basal application from T_2 to T_{10} treatments.*

3.4 Relative Growth Rate

The results shown that application of 75 % recommended dose of NP +100 % K along with soil application of LCO fortified bio-fertilizer @ 10 kg ha^{-1} + foliar application of 19:19:19 @ 2% spray at flowering and pod filling stages has recorded significantly higher relative growth rate during 30 to 60 DAS and 60 DAS to at harvest (0.040 and 0.031 g g^{-1} day^{-1}, respectively) and it was on par with treatment T_6 *i.e.,* 100 % RDF along with foliar application of WSF 19:19:19 @ 2% spray at flowering and pod filling stages (0.039 and 0.028 g g^{-1} day^{-1}, respectively). the growth promotion of plants inoculated with microbial secondary metabolites could be at least partially related to the fact that LCOs indirectly affect photosynthesis and accelerated growth by stimulating mitotic activity in the meristematic tissue of leaves [8].

4. CONCLUSION

The efficacy of nutrients provided by soil application is frequently reduced as a result of leaching and volatilization losses. Specific to soybean, leaf withering begins far before pod maturity is complete, breaking the source-sink interaction and ultimately resulting in empty pods and pods with shriveled seeds. These problems can be resolved by applying nutrients topically to the plant.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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