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Nod Factor (Lipo Chito-Oligosaccharide) and its Impact on Nutrient Uptake in Maize (*Zea mays*)

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ABSTRACT: *The uptake of nitrogen, phosphorus and potassium by plants was found higher in Lipo Chito-Oligosaccharide (LCO) @ 300 ml ha^{-1} at 20-25 days after sowing (DAS) (V_2) and 50-55 DAS (V_4) stages treatment at all stages of crop growth after the application of LCO in maize. Soil N, P and K contents were reduced in LCO @ 300 ml ha^{-1} at V_2 and V_4 stages at post cropping period compared to pre cropping period. Increased nutrient uptake by plants inoculated with bacterial formulated LCO was attributed to the production of plant growth regulators (viz., auxin and cytokinin) by the bacteria at the root interface, which stimulated root development that resulted in better absorption of water and nutrients from the soil. The response of maize to different concentrations of LCO showed significant increases in 'N' uptake at all growth stages and the increment was 7.0 per cent, whereas for phosphorous, the uptake increase was 2.5 per cent increase in the potassium uptake was 6.4 per cent.*

Keyboard: *Lipo Chito-Oligosaccharide, Nitrogen, Phosphorus, Potassium, and Nutrient Uptake*

INTRODUCTION

Lipo-Chito Oligosacchrides (LCO), in general, are capable of influencing plant growth and development. They are mainly secreted by Rhizobia spp. and play a key role in the stimulation of nodules in legumes by activating signaling events of certain growth hormones, nutrients and important plant metabolism. LCO have both direct and indirect effects on various physiological processes. They provide minerals, biochemical substances and nutrient to the rhizosphere microbial population, carry the trace elements and growth regulators for stimulating plant growth. Lipo-chito oligosaccharides (LCOs), secreted by rhizosphere bacteria, play a key role in the stimulation of nodules in the roots of legumes by activating signaling events that, at very low concentration, initiate cell division at distinct sites in the root (Lerouge *et al*., 1990; Truchet *et al*., 1991).

The structure of LCOs from different rhizobia has been determined as an oligomer of three to five â-1,4 linked molecules of N-acetyl-D-glucosamine with additional substitution on the terminal sugar residues (Denarie and Cullimore, 1993). Some strains of rhizobia synthesize a large number of LCOs. The LCOs produced by *B. japonicum* are pentameric molecules with C18:1, C16:1 and C16:0 fatty acid chains at the non-reducing end, and 2-0 methylfucose at the reducing end of the chitin backbone (Carlson *et al*., 1993).

MATERIALS AND METHODS

The experiments were conducted at Department of Crop Physiology, Field No. NA7 of Eastern Block, Tamil Nadu Agricultural University, Coimbatore in Rabi (September 2011 to December 2011) and Summer (February 2012 to May 2012). The experimental field is located at 11°E longitude and 77°N latitude in an altitude of 426.8 m above mean sea level to investigate the efficacy of Lipo - Chito oligosaccharides (LCO) on nutrient uptake in maize. The treatments contain foliar application of LCO and seed treatment of biofertilizers (*Azospirillum, Rhizobium and Phosphobacteria*) and biocontrol agents *viz., Pseudomonas and Trichoderma*. A control was maintained with the recommended dose of inorganic fertilizers. LCO at different concentrations (LCO @ of 150 ml ha^{-1}, 300 ml ha^{-1} and 600 ml ha^{-1}) were applied at two stages: V_2 stage (Maize – 20-25 DAS) and V_4 stage (Maize – 50-55 DAS).

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Ratchet is a LCO promoter compound which has been manufactured by EMD Crop Biosciences. It contains a minimum of 4 x 10^{-7}% lipo-chito oligosaccharide (LCO) in an aqueous carrier, which has been formulated for maize and soybean as foliar application. This product contains only components that are naturally occurring in soil and are biodegradable. Ratchet is compatible with most of the post emergence applied products such as pesticides and to get an optimal result, it has to be applied between V_2 - V_6 stages of the crop.

NUTRIENT ANALYSIS IN SOIL AND PLANT UPTAKE

Soil analysis

Pre-experiment composite soil samples were collected and analysed for mechanical and chemical properties. Postharvest samples were collected from each treatment plot and analysed for the following nutrients:

S. No.	*Nutrient*	*Methodology*
1.	Available N	Alkaline KMnO4 method (Subbiah and Asija, 1956)
2.	Available P_2O_5	Olsen's method (Olsen *et al*., 1954)
3.	Available K 2O	Ammonium acetate method (Stanford and English, 1949)

Plant analysis

The plant samples were collected at seedling (15-20 DAS), vegetative (30-35 DAS), flowering (60-65 DAS) and maturity (90-95 DAS) stages in maize. The samples were ground into fine powder in a Willey mill and used for chemical analysis for NPK nutrient contents using the following methods. The uptake of nutrients were calculated by multiplying the nutrient content in dry matter and expressed in kg ha^{-1}.

S. No.	*Nutrient*	*Methodology*
1.	Total Nitrogen	Microkjeldahl (Humphries, 1956)
2.	Total Phosphorus	Triple acid digestion spectrometer (Jackson, 1973)
3.	Total Potassium	Triple acid digestion Flame photometer (Jackson, 1973)

RESULTS

Nutrients availability

Available nutrients in soil (kg ha^{-1})

Both pre-planting and post-experimental soil samples were collected and analyzed for NPK. The initial soil samples did not show any variation for available N, P and K.

In postharvest soil samples, N, P and K contents were found to decrease considerably compared to the initial soil samples. Considering the effect of LCO treatment, irrespective of both the seasons, the increase in LCO treatment reduced the available N, P and K contents compared to untreated control.

The available N in the final soil sample was considerably low compared to initial soil sample. The effect of LCO treatment showed an evident variation compared to untreated control. The maximum available N values (173.9, 163.5) were in T_1 and the lowest in T_{11} (172.3, 165.3) **(Table 1)**. Both pre-sowing and post-experimental soil samples were collected and analyzed for NPK. The initial soil samples did not show any variation for available N, P and K.

In postharvest soil samples, N, P and K contents were found to reduce considerably compared to the initial soil samples. Considering the effect of LCO treatment, irrespective of both the seasons, the increase in LCO treatment decrease the available N, P and K contents compared to untreated control.

The available N in the final soil sample was considerably low compared to initial soil sample. The effect of LCO treatment exhibited clear variation compared to untreated control by registering the maximum available N content (41.00, 36.11) in T_1 and the minimum in T_9 (34.05, 31.82) **(Table 1.)**.

The initial soil sample did not show any difference in available P **(Table 2.)**. Considering the effect of LCO on P content, the spray at two different stages reduced the soil P compared to control and the maximum P contents were found in T_1 (3.05, 2.68) and the minimum in T_9 (3.09, 2.85).

The available potassium in initial sample didn't show any variation, but in postharvest soil sample, reduction in K content was observed. Among the treatments, the lowest value was observed in T_7 (214.68, 185.11) **(Table 3)**.

DISCUSSION

An enhanced plant 'nitrogen uptake' by Rhizobium is has been noticed by Bertrand *et al*. (2000). The increase in the amount of nutrient taken up in Rhizobium – inoculated plants was due to increase in root surface, and more uptake of NO^{3-} to meet the plant's demand and a similar trend of results noticed by Imasand and Touraine, (1994) in lettuce strongly supports the trend obtained now.

Nitrogen is the mineral element that is required by plants in higher amounts. It serves as a constituent

Table 1
Effect of lipo- chito oligosaccharides (LCO) on available Nitrogen (kg ha^{-1}) in soil

Treatments	*Initial*		*Initial*	
	S_1	S_1	S_1	S_1
T_1 – Control	169.08	157.24	41.00	36.11
T_2 - *Pseudomonas fluorescens* seed treatment @ 10g kg^{-1}	170.87	158.91	36.91	31.85
T_3 - *Trichoderma viride* seed treatment @ 4g kg^{-1}	172.34	160.28	39.44	37.90
T_4 - *Rhizobium* + *Phosphobacteria* seed treatment @ 600g ha^{-1}	169.61	157.73	34.69	31.80
T_5 - LCO @ 150ml ha^{-1} at V_2 stage	170.00	158.10	33.62	32.67
T_6 - LCO @ 300ml ha^{-1} at V_2 stage	170.76	158.81	34.52	31.50
T_7 - LCO @ 600ml ha^{-1} at V_2 stage	171.71	159.69	34.28	33.14
T_8 - LCO @ 150ml ha^{-1} at V_4 stage	172.40	160.33	36.13	34.80
T_9 - LCO @ 300ml ha^{-1} at V_4 stage	172.30	160.24	34.05	31.82
Mean	171.01	159.04	36.07	33.51

*V_2 = 20 -25 DAS; V_4 = 40 – 45 DAS

Table 2
Effect of lipo- chito oligosaccharides (LCO) on Available Phosphorous (kg ha^{-1}) in soil

Treatments	*Initial*		*Final*	
	S_1	S_2	S_1	S_2
T_1 – Control	23.62	20.72	3.05	2.68
T_2 - *Pseudomonas fluorescens* seed treatment @ 10g kg^{-1}	23.85	20.95	3.08	2.70
T_3 - *Trichoderma viride* seed treatment @ 4g kg^{-1}	22.25	21.23	2.87	2.74
T_4 - *Rhizobium* + *Phosphobacteria* seed treatment @ 600g ha^{-1}	22.34	21.42	2.88	2.77
T_5 - LCO @ 150ml ha^{-1} at V_2 stage	22.62	21.72	2.92	2.81
T_6 - LCO @ 300ml ha^{-1} at V_2 stage	22.92	21.92	2.96	2.83
T_7 - LCO @ 600ml ha^{-1} at V_2 stage	23.14	22.00	2.99	2.84
T_8 - LCO @ 150ml ha^{-1} at V_4 stage	23.30	21.88	3.01	2.82
T_9 - LCO @ 300ml ha^{-1} at V_4 stage	23.35	22.10	3.01	2.85
Mean	23.04	21.55	2.97	2.78

*V_2 = 20 -25 DAS; V_4 = 40 – 45 DAS

Table 3
Effect of lipo- chito oligosaccharides (LCO) on Available Potassium (kg ha^{-1}) in soil

Treatments	*Initial*		*Final*	
	S_1	S_2	S_1	S_2
T_1 – Control	372.95	346.84	224.95	193.99
T_2 - *Pseudomonas fluorescens* seed treatment @ 10g kg^{-1}	372.04	346.00	222.35	191.56
T_3 - *Trichoderma viride* seed treatment @ 4g kg^{-1}	371.99	345.95	220.29	191.33
T_4 - *Rhizobium* + *Phosphobacteria* seed treatment @ 600g ha^{-1}	371.38	345.39	218.36	190.16
T_5 - LCO @ 150ml ha^{-1} at V_2 stage	371.14	345.16	216.00	187.97
T_6 - LCO @ 300ml ha^{-1} at V_2 stage	372.18	346.12	215.55	186.88
T_7 - LCO @ 600ml ha^{-1} at V_2 stage	371.88	345.85	214.68	185.11
T_8 - LCO @ 150ml ha^{-1} at V_4 stage	371.99	345.95	215.65	184.08
T_9 - LCO @ 300ml ha^{-1} at V_4 stage	372.07	346.02	214.18	183.77
Mean	371.96	345.92	218.00	188.32

*V_2 = 20 -25 DAS; V_4 = 40 – 45 DAS

of many plant cell components, including amino acids and nucleic acids. The response of maize to different concentrations of LCO showed a significant increase in 'N' uptake at all growth stages and the increment was 7.0 per cent. It is generally assumed that LCO application enhances the Rhizobium population which triggers an increase in root surface area which inturn increased mineral uptake and, shoot biomass accumulation.

Rhizobium inoculation significantly increased nitrogen content of lablab bean (Mohamed Ahmed *et al*., 2011). Increased nutrient uptake by plants inoculated with bacteria was attributed to the production of plant growth regulators by the bacteria as a symbiotic event which stimulated root development and resulted in better absorption of water and nutrients from the soil (Hoflich and Kuhn, 1996). Increased uptake of nutrients such as N, P and K was suggested by one of the mechanisms by which PGPR increased crop yield (Kapulnik *et al*., 1985).

The enhancement of total nitrogen uptake by Rhizobium might be assigned to more availability of nitrogen for uptake, enhanced cation exchange capacity of roots due to better root proliferation and besides, to higher dry matter accumulation and grain yield (Utpal and Bandopadhyay, 2004). This could be due to more build up of nitrogen in the grain and straw which might have increased phosphorus and potassium contents in grain and straw and their uptake (Singh *et al*., 1993). Besides, Hamdia *et al.* (2004) reported that *Azospirillum brasilense* inoculation increased N content of maize under salt stress and it could be explained by N2 fixing ability and increased NR (nitrate reductase) and NA (nitrogenase) activities of *A. brasilense*.

Phosphorus (P) is a major growth-limiting nutrient, and unlike the case for nitrogen, there is no large atmospheric source that can be made biologically available (Ezawa *et al*., 2002). Root development, stalk and stem strength, flower and seed formation, crop maturity and production, N-fixation in legumes, crop quality, and resistance to plant diseases or abiotic stress are the attributes associated with phosphorus nutrition. The uptake of phosphorus was significantly increased by the application of LCO and a maximum increase of 2.5 per cent in maize was established. Useful compounds like lipo-oligosaccharides and growth hormones produced by rhizobia lowers the enzymes ACC deaminase, providing bio-available phosphorous for plant uptake, sequestering iron for plant by siderophores (Mehboob *et al*., 2009). In general, plants appear to be remarkably efficient in their internal recycling of phosphorus. Phosphorus uptake by the plants was increased due to the enhanced activity of acid phosphatase (Djanaguiraman, 2003), by solubilising organic phosphates in the rhizosphere (Goldstein *et al*., 1989). Phosphate is relatively immobile and it is present in very low concentration in soil solution. So, substantial amount of phosphate fertilizers are applied to agricultural soils.

The increased nitrogen could have also increased the potassium availability through its synergistic effect. Similar results were obtained by Srinivasan Rao *et al*. (1997). Lal *et al*. (2000) reported that microorganisms and organic decomposition affects the availability of potassium through liberation of organically bound potassium from insoluble form present in soil and minerals (**Fig. 1)**.

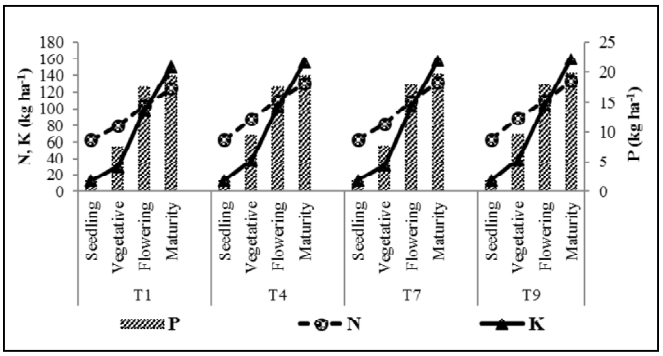

Figure 1: Effect of LCO on NPK uptake (kg ha^{-1}) of maize at different stages

Application of LCO could increase the potassium uptake to a maximum of 6.4 per cent in maize. Potassium uptake is significantly increased from vegetative stage to harvest stage of the crop growth. Potassium accumulation in the leaves was found higher by bacterial inoculation. The preferential K accumulation in the leaves, as a result of bacterial inoculation, might also be related to changes in membrane activity and subsequently proton efflux in roots (Bashan *et al*., 1991).

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