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# Zinc foliar spray on snap beans using nano-Zn with N-soil application using mineral, organic and biofertilizer

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## ABSTRACT

Two factorial field experiments on snap beans (*Phaseolus vulgaris* L.) grown on a heavy clay soil, were executed in 2 seasons (2015 and 2016). Zn-spray with 4 treatments of No-Zn spray (water spray) ($Zn_0$), Zn-sulphate ($Zn_1$), Zn-chelate (Zn.DTPA) ($Zn_2$) and nano-ZnO ($Zn_3$). Zn-solutions contained 50 mg Zn $L^{-1}$. N was applied through soil at 144 kg N $ha^{-1}$ as mineral ammonium sulphate ($N_1$), 72 N $ha^{-1}$ as mineral + 72 N $ha^{-1}$ as organic (compost) ($N_2$); and 72 N $ha^{-1}$ mineral + 72 N $ha^{-1}$ as organic + biofertilizer *(Rhizobium)* ($N_3$). Non-sprayed plants gave 12.9 Mg $ha^{-1}$ fresh pods. Traits of plant height, No. of leaves and leaf area $plant^{-1}$ increased due to Zn-spry. Increases by $Zn_1$, $Zn_2$ and $Zn_3$ were 6.8, 8.3 and 11.9 % respectively for height, 8.6, 7.6 and 18.3%, for leaf area and 8.3, 22.4 and 29.6% for pod yield. The $N_2$ or $N_3$ treatments gave greater positive response than the $N_1$ treatment.

***Key words:*** Nano ZnO, Zn spray, Snap bean, compost and biofertilizer

## Introduction

Legume crops have exceptional properties among edible plants. They are the major source of plant proteins, edible oils and minerals (Márquez-Quiroz *et al*., 2015) as well as vitamins, and antioxidants (Katoch, 2013). Snap bean (*Phaseolus vulgaris* L.) is an important legume crop, which - besides being a source of protein and minerals- is a source of chemical substances of useful medicinal properties (Jha *et al*., 2015). Since pollution of environment is becoming a great concern involving fertilization practices, using friendly materials are gaining grounds in agricultural management (Tiwari, 2002). In actual practice, the use of chemical fertilizers cannot be completely abandoned, and their rationalized use with organic and biological fertilizers may offer a compromise solution to this situation. Organic manures in combination with mineral chemical fertilizers can be used with reasonable success in the long run (Swarup, 1998).

Nutrients can be given to plants in several forms of organic fertilizers, though not as readily available as the soluble chemical ones (Naeem *et al*., 2006). Besides their role in increasing soil fertility by enriching the soil with available nutrients, organic manures can enhance soil fertility through improving soil physical conditions (Haynes, and Naidu, 1998 and Dauda *et al*., 2008). Organic matter in soil can increase through organic fertilization, and this encourages the useful activities of soil microorganisms (El-Gizy, 1994), through increasing mineralization of organically bound nutrients such as N or P (Marschner, 2012). Reinforcing fertility in the rhizosphere through inoculants of useful microorganisms .i.e. biofertilizers facilitate and perform operations which render unavailable plant nutrients into available forms. Examples of such biofertilizers are $N_2$-fixers and P-

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dissolvers (Abdel-Salam 2014). Many micronutrients are needed for plants and humans, Zinc is one of such micronutrients since it activates the functions of many enzymes such as aldolases, transphosphorylases and processes such as cell division, protein synthesis and maintenance of membrane structure (Andreini *et al*., 2006, Broadley *et al*., 2007 and Marschner, 2012). Application of plant nutrients, particularly micronutrients, through foliar spray proved practical for many crops and can result in a quick positive response and an economizing in application rates (Fernández *et al*, 2013 and Sida-Arreola *et al*., 2017). It may help in avoiding the harmful effect of high doses of some micronutrients applied through the soil (Obreza *et al*., 2010).

Delivering plant nutrients through nanotechnology methods are becoming an effective management in plant nutrition (Solanki *et al*., 2015 and Ghorbanpour *et al*., 2017). This technology involves manufacturing fertilizer materials formulating them into extremely small minute particles (nano-particles of < 100 nm Ø) and apply to plants to improve their utilization and efficiency (Rai *et al*., 2015). Such technology is adopted in many fields besides fertilizer application. Some difficulties were encountered in the use of nano-technologies (Selivanov and Zorin, 2001 and Scott and Chen, 2003). Nano-fertilization has advantages including adjusted nutrient release, decreased environmental pollution (Naderi *et al*., 2011, Batsmanova *et al*., 2013 and Ghorbanpour *et al*., 2017). Favourable effects of nano-Zn application include creation of active Zn phosphate inside the plant; and conversion to Zn-phosphates and ZnO which exist on plant cell tips (Lv *et al*., 2015). Also it hastens seed germination (Prasad *et al*., 2012) and other positive effects (Subramanian and Sharmila-Rahale, 2012, Ghorbanpour *et al*., 2017 and Singh *et al*., 2013). Increases in fruit diameter have been attributed to the role of Zn on the synthesis of tryptophan and auxin (Scott, 2008 and Swietlik, 1999). Sprays of nano ZnO on Spinach (*Spinacia oleracea*) at 50 to 100 mg $L^{-1}$ caused increases in a number of traits including leaf length, width, surface area, chlorophyll and fibre as well as protein contents (Kisan *et al*. 2015). Peanut pod yield increased by 30% with nano ZnO spray (Prasad *et al*., 2012).

The current study was aimed at assessing the effect of foliar-spraying of Zn in forms of sulphate, chelate, and nano-ZnO to snap bean (*Phaseolus vulgaris* L.) with different sources of nitrogen.

## Materials and Methods

Two field experiments on snap beans (*Phaseolus vulgaris* L.; cv River-Grow) grown on a heavy clay soil (Table 1) were carried out during 2015 and 2016 seasons in the Research farm of the Faculty of Agriculture, Moshtohor, Benha University, Qalubia Governorate, Egypt to assess the response of Zn foliar spray using the nanotechnology methodology under conditions of N- mineral, organic and bio-fertilization. The Zn sources were Zn-sulphate, Zn-chelate (Zn.DTPA) and ZnO, Soil physical and chemical properties were determined according to Jackson (1973) and Black (1982).

**Table 1:** Physical and chemical properties of soil of the experiment.

| Particle size distribution and Soil texture | | | | pH | EC* ($dS\ m^{-1}$) | OM ($gkg^{-1}$) | CaCO3 ($gkg^{-1}$) | Available N,P&K* ($mg\ kg^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sand | Silt | Clay | Texture* | | | | | | | |
| % | | | | | | | | N | P | K |
| 24.4 | 24.6 | 51 | Heavy clay | 7.9 | 2.16 | 1.41 | 1.53 | 22.5 | 9.1 | 120 |

**Texture according to the international soil texture Triangle (Moeys 2016) ; EC of paste extract ; Extractants are KCl(N) , $NaHCO_3$(P) , $NH_4Ac$ (K)*

The experimental design was a split plot with four replicates. The factors and their treatments were as follows. **Factor A (main plots): N application** with 3 treatments of $N_1$, $N_2$ and $N_3$; i.e. 144 kg N $ha^{-1}$ as mineral, 72 kg N as mineral + 72 kg N $ha^{-1}$ as organic N and 72 kg N as mineral + 72 kg N as organic $ha^{-1}$ + biofertilizer (*Rhizobium*) respectively. **Factor B (sub plots): Zn-foliar spray** with 4 treatments of $Zn_0$, $Zn_1$, $Zn_2$ and $Zn_3$; i.e. no-Zn (spray with distilled water), spray with $ZnSO_4$, Zn-DTPA and nano-ZnO, respectively. The mineral fertilizer was ammonium sulphate (210 g N $kg^{-1}$) and the organic was compost (Table 2) while the biofertilizer was inocula of *Rhizobium* bacteria. The 144 kg N $ha^{-1}$ is a rate recommended for snap beans in the region. The total number of treatment combinations was 12 (3 N treatments x 4 Zn treatments).

Concentration of the spray solution was 50 mg Zn $L^{-1}$. Plants received 3 sprays: the first was given 21 days after seeding, the second and third were given 10 and 20 days respectively after the first.

Bacterial strain of rhizobia (*Rhizobium leguminosarum biovar phaseoli*) was obtained from Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. The biofertilizer suspension was given through the soil in two doses: the first was with the compost and the second was added to soil 14 days after seeding. The nano-ZnO (<50 nm Ø) was provided by Nano-technology Laboratory, Environmental Studies Research Institute (ESRI), EL-Sadat City University, Egypt.

The experimental plot consisted of five ridges 3.5-m long and 60 cm wide (an area of 10.5 $m^2$) Regular agricultural practices were carried out as followed in the region. Seeds (obtained from Suez Canal Trade & Agricultural Development Co.) were sown on 14th of September in each season on one side of the ridges in hills 15-cm apart. Thinning was done after complete seed germination (21 days after seeding) and two plants per hill were left.

**Table 2:** Main properties of the compost used in the study.

| Parameter | Value |
|---|---|
| pH | 8.11 |
| EC (1:5 extract) (dS m-1 ) | 8.21 |
| Organic matter (g kg-1) | 216 |
| Organic-C (g kg-1) | 125.4 |
| Total-N (g kg-1) | 12.1 |
| C:N ratio | 10.4 |
| Total-P (g kg-1) | 9.1 |
| N-$NH_4$ (mg kg-1 )* | 175 |
| N-$NO_3$ (mg kg-1)* | 50 |

**: Extracted by KCl*

## Data recorded.

*Vegetative growth traits.*

At full blooming stage (60 days after seeding) five plants from each plot were taken and the following traits were recorded: plant height, number of leaves $plant^{-1}$, leaf area $plant^{-1}$, fresh weight $plant^{-1}$and dry weight $plant^{-1}$. Fresh samples were taken then dried at 70ºC for 72 hrs.

*Green pods yield.*

Plant green pods were harvested at maturity stage and weighed.

*Pod quality.*

A representative sample of 10 green pods was taken and dried at 70ºC till constant weight and taken for analysis. N, P and K in pods were determined according to Pregl (1945), John (1970) and Brown and Lilleland (1946); Zn was determined as described by Chapman and Pratt (1961); total carbohydrates, it was determined according to Herbert *et al*. (1971). A factor of 6.25 was used for conversion of total nitrogen to protein percentage.

*Statistical analysis:*

The obtained data in both seasons of study were subjected to analysis of variance as a factorial experiment in split plot design. L.S.D. method was used to differentiate between means according to Snedecor and Cochran (1991).

## Results and Discussion

### Growth characteristics Plant height, number of leaves , leaf area, fresh and dry weight, (Tables 3 and 4).

Plant height of the non-sprayed was lower than the sprayed (Table 3), and ranged from 46.30 to 50.52. Spraying of Zn caused increases in plant height averaging 6.8, 8.3 and 11.9 % due to $Zn_1$, $Zn_2$ and $Zn_3$ respectively. The highest positive effect caused by the nano-Zn is a demonstration of the superiority of this method in enhancing plant growth. Subramanian and Sharmila-Rahale (2012) used nano Zn loaded on zeolite clay mineral and obtained increased growth of lettuce plant.

Differences between the three N treatments in the current study, were not significant in the first season, but in the second season, the $N_3$ treatment (which included the Rhizobium biofertilizer combined with 72 kg mineral N+72 organic N $ha^{-1}$) gave the highest plant height, thus demonstrating the enhancing effect of combining mineral and organic N with the N-biofertilizer.

**Table 3:** Effect of Zinc foliar spray on snap beans using nano-Zn with N soil application of mineral, organic and biofertilizer: Plant height, No. of leaves and fresh weight.

| Zn-Spray (Zn) | N-Application (N) | | | | N-Application (N) | | | | N-Application (N) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $N_1$ | $N_2$ | $N_3$ | Mean | $N_1$ | $N_2$ | $N_3$ | Mean | $N_1$ | $N_2$ | $N_3$ | Mean |
| | **Plant height (cm)** | | | | **No. of leaves/plant** | | | | **Total fresh weight ( g $plant^{-1}$)** | | | |
| | **First season (2015)** | | | | | | | | | | | |
| $Zn_0$ | 46.77 | 48.94 | 46.30 | 47.34 | 16.62 | 19.38 | 16.32 | 17.44 | 106.6 | 111.3 | 125.3 | 114.4 |
| $Zn_1$ | 50.27 | 50.50 | 52.23 | 51.00 | 21.14 | 19.07 | 18.22 | 19.48 | 127.2 | 128.7 | 128.0 | 127.9 |
| $Zn_2$ | 52.53 | 52.83 | 52.60 | 52.66 | 20.27 | 19.05 | 21.69 | 20.34 | 134.1 | 135.1 | 138.0 | 135.7 |
| $Zn_3$ | 52.57 | 55.07 | 55.40 | 54.35 | 17.95 | 23.07 | 25.40 | 22.14 | 140.2 | 152.3 | 160.7 | 151.1 |
| mean | 50.53 | 51.84 | 51.63 | | 18.99 | 20.14 | 20.41 | | 127.0 | 131.9 | 138.0 | |
| LSD $_{0.05}$ | N: ns Zn: 2.48 NZn: 4.29 | | | | N: 2.07 Zn: 1.47 NZn: 2.55 | | | | N: 3.4 Zn: 5.2 NZn: 9.1 | | | |
| | $N_1$ | $N_2$ | $N_3$ | Mean | $N_1$ | $N_2$ | $N_3$ | Mean | $N_1$ | $N_2$ | $N_3$ | Mean |
| | **Plant height (cm)** | | | | **No. of leaves/plant** | | | | **Total fresh weight (g $plant^{-1}$)** | | | |
| | **Second season (2016)** | | | | | | | | | | | |
| $Zn_0$ | 50.52 | 47.55 | 50.30 | 49.46 | 19.60 | 19.53 | 20.63 | 19.92 | 110.2 | 115.4 | 115.7 | 113.8 |
| $Zn_1$ | 50.13 | 54.97 | 52.18 | 52.42 | 20.68 | 20.53 | 22.05 | 21.09 | 119.3 | 119.3 | 121.4 | 120.0 |
| $Zn_2$ | 52.46 | 50.51 | 53.63 | 52.20 | 19.56 | 19.68 | 20.34 | 19.86 | 121.4 | 122.5 | 122.9 | 122.3 |
| $Zn_3$ | 52.68 | 53.97 | 55.40 | 54.01 | 20.89 | 22.53 | 22.73 | 22.05 | 125.2 | 126.1 | 127.5 | 126.3 |
| Mean | 51.44 | 51.75 | 52.88 | | 20.18 | 20.57 | 21.44 | | 119.0 | 120.8 | 121.9 | |
| LSD $_{0.05}$ | N: 0.94 Zn: 1.67 NZn: 2.89 | | | | N: ns Zn: ns NZn: ns | | | | N: 3.6 Zn: 4.3 NZn: 7.5 | | | |

*Notes: $N_1$, $N_2$ and $N_3$ are 144 kg mineral N $ha^{-1}$ , 72 organic N$ha^{-1}$+72 kg mineral N $ha^{-1}$ and 72 organic N $ha^{-1}$ + 72 kg mineral N $ha^{-1}$+biofertilizer (Rhizobium); $Zn_0$ $Zn_1$ $Zn_2$ and $Zn_3$ are no Zn (spray with distilled water), spray with $ZnSO_4$, Zn-DTPA and Nano-ZnO. (mineral N is ammonium sulphate, organic N is compost)*

The number of leaves per plant followed a pattern of response rather similar to that of plant height (Table 3). The number of leaves plant$^{-1}$ increased by application of Zn. The non-sprayed plants showed fewer leaves than the sprayed, exhibiting from 16.3 to 20.63 leaves plant$^{-1}$ compared with 17.94 to 25.40 given by the Zn-sprayed plants during the two seasons. The increase in the number of leaves plant$^{-1}$ caused by the Zn-treatments averaged 8.6, 7.6 and 18.3% due to $Zn_1$, $Zn_2$ and $Zn_3$ respectively. The marked efficiency of the nano-Zn d in increasing the number of leaves is most probably due to enhancing P-mobilizing enzymes along with other growth improving properties (Raliya and Tarafdar 2013). As for the pattern of the differences between the different N treatments, results were comparable to those regarding plant height. Differences between N treatments were not significant in the first season but in the second one the $N_3$ treatment (72 kg mineral N + 72 kg organic N ha$^{-1}$ + biofertilizer) proved the most effective one.

Fresh weight plant$^{-1}$ increased upon spraying with Zn (Table 3). The increase was considerable, particularly with the nano-Zn spray. Non-sprayed plants gave fresh weight (g plant$^{-1}$) of 106.6 to 125.20 compared with 119.3 to 160.7 given by the Zn-sprayed plants during the two seasons.

Highest response occurred with the nano-Zn spray. The increase in the total fresh weight plant$^{-1}$ by the Zn treatments averaged 8.6, 13.1 and 21.2 % due to $Zn_1$, $Zn_2$ and $Zn_3$ respectively. The positive response to Zn spray, particularly to the nano-Zn spray, was very much consistent with regard to all of the above mentioned traits in both seasons. The pattern of dry weight plant$^{-1}$ (Table 4) was nearly similar to that of the fresh weight. The positive response to nano-Zn application on plant growth was reported by a number of researchers (Prasad *et al*., 2012 Subramanian and Sharmila-Rahale 2012, Raliya and Tarafdar 2013 and Kisan *et al*., 2015).

Leaf area plant$^{-1}$ increased by Zn-foliar spray (Table 4). The increase was more marked with nano-Zn. Increases averaged 0.7, 3.8 and 9.8%due to sulphate-Zn, DTPA-Zn and nano-Zn respectively.

**Yield of pods (Table 4)**

Lowest pod yield was 12.89 Mg ha$^{-1}$ given by the non-spayed plants which received the full rate of N as mineral. The highest yield was given by $N_3$ treatment (72 kg mineral N+72 organic N ha$^{-1}$ + biofertilizer) which showed an increase of 33.4% over that of the lowest. This agrees with finding of Shams (2012) who reported that using of 50% of the mineral N + 50% of the organic N and inoculation with nitrogen-fixing bacteria, gave the best results in the total yield of Kohlrabi as compared with the control (100% mineral N). The superiority of adding 50% of N in the mineral form + 50% of N in the organic form over the other N-fertilizer systems may be referred to the increase in microorganisms activity and increasing adsorbing capacity of essential nutrients against leaching. Moreover adding mineral + organic fertilizer together will improve the mineralization of organic-N (Tisdale and Nelson, 1975). All treatments which received Zn-foliar spray exhibited increased yield over the non-sprayed. Average increases for the two seasons were 8.3, 22.4 and 29.6% for $Zn_1$, $Zn_2$ and $Zn_3$ respectively; the nano-Zn gave the highest response. Foliar spray of many crops using Zn, particularly the nano-Zn, proved effective and increased yield (Subramanian and Sharmila-Rahale 2012, Razzaq *et al*. 2013).

**Pod quality "protein and carbohydrate contents"(Table 5)**

Contents of protein and carbohydrates in pods were not much affected by Zn-spray. Spraying of Zn in both seasons had no significant effect on protein content in pods (Table 5). However with regard of N treatments, a difference between the three treatments were not significant in the first season, but was significant in the second season. The N3 treatment which included the Rhizobium biofertilizer combined with 72 kg mineral N + 72 organic N ha-1 gave the highest protein content. The increase of protein content due to $N_3$ treatment could be attributed to the N fixation by biofertilizer (*Rhizobium phaseoli*) thus supplying snap beans with it leading to higher protein content (Elbanna *et al*., 2009 and Çiğdem, 2011).

**Table 4:** Effect of Zinc foliar spray on snap beans using nano-Zn with N soil application of mineral, organic and biofertilizer: Dry weight, leaf area and pods fresh yield.

| Zn-Spray (Zn) | N-Application (N) | | | | N-Application (N) | | | | N-Application (N) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $N_1$ | $N_2$ | $N_3$ | Mean | $N_1$ | $N_2$ | $N_3$ | Mean | $N_1$ | $N_2$ | $N_3$ | Mean |
| | **Total dry weight (g plant$^{-1}$)** | | | | **Leaf area plant$^{-1}$ (cm$^2$)** | | | | **Pod fresh yield (Mg ha$^{-1}$)** | | | |
| | **First season (2015)** | | | | | | | | | | | |
| $Zn_0$ | 18.22 | 19.71 | 19.72 | 19.22 | 757.7 | 863.2 | 874.0 | 831.6 | 12.89 | 12.42 | 13.17 | 12.83 |
| $Zn_1$ | 18.40 | 19.73 | 20.39 | 19.50 | 906.3 | 865.1 | 925.3 | 898.9 | 13.03 | 13.31 | 14.30 | 13.55 |
| $Zn_2$ | 20.33 | 20.17 | 20.13 | 20.21 | 918.8 | 829.2 | 932.4 | 893.5 | 15.63 | 13.96 | 17.00 | 15.53 |
| $Zn_3$ | 20.43 | 22.20 | 22.53 | 21.72 | 932.4 | 1087.9 | 1115.3 | 1045.2 | 15.55 | 17.02 | 17.20 | 16.59 |
| Mean | 19.3 | 20.5 | 20.7 | | 878.8 | 911.4 | 961.7 | | 14.28 | 14.18 | 15.42 | |
| LSD $_{0.05}$ | N: 0.30 Zn: 0.68 NZn: 1.17 | | | | N: 35.5 Zn: 59. 6 NZn: 103.2 | | | | N: ns Zn: 0.39 NZn: 0.68 | | | |
| | $N_1$ | $N_2$ | $N_3$ | Mean | $N_1$ | $N_2$ | $N_3$ | Mean | $N_1$ | $N_2$ | $N_3$ | Mean |
| | **Total dry weight (g plant$^{-1}$)** | | | | **Leaf area plant$^{-1}$ (cm$^2$)** | | | | **Pod fresh yield (Mg ha$^{-1}$)** | | | |
| | **Second season (2016)** | | | | | | | | | | | |
| $Zn_0$ | 18.68 | 18.63 | 19.93 | 19.08 | 842.1 | 872.1 | 919.8 | 878.0 | 11.76 | 11.17 | 11.32 | 11.42 |
| $Zn_1$ | 19.05 | 18.83 | 19.28 | 19.06 | 795.8 | 819.5 | 859.0 | 824.8 | 13.03 | 12.24 | 12.93 | 12.73 |
| $Zn_2$ | 19.29 | 19.34 | 20.03 | 19.55 | 877.8 | 847.4 | 886.0 | 870.4 | 14.14 | 12.71 | 15.63 | 14.16 |
| $Zn_3$ | 20.01 | 20.27 | 20.74 | 20.34 | 863.8 | 913.1 | 954.7 | 910.5 | 14.12 | 14.64 | 15.77 | 14.84 |
| Mean | 19.26 | 19.27 | 19.99 | | 844.9 | 863.0 | 904.9 | | 13.26 | 12.69 | 13.91 | |
| LSD $_{0.05}$ | N: ns Zn: 0.54 NZn: 0.93 | | | | N: ns Zn: 40.6 NZn: 70.3 | | | | N: 0.15 Zn: 0.29 NZn: 0.49 | | | |

*Notes: see footnote of Table 3 for treatment designation*

**Table 5:** Effect of Zinc foliar spray on snap beans using nano-Zn with N soil application of mineral, organic and biofertilizer: protein and carbohydrate in pods.

| Zn-Spray (Zn) | N-Application (N) | | | | N-Application (N) | | | |
|---|---|---|---|---|---|---|---|---|
| | $N_1$ | $N_2$ | $N_3$ | Mean | $N_1$ | $N_2$ | $N_3$ | Mean |
| | **Protein content in pods (g kg$^{-1}$)** | | | | **Carbohydrate content in pods (g kg$^{-1}$)** | | | |
| | **First season (2015)** | | | | | | | |
| $Zn_0$ | 138.3 | 152.6 | 135.4 | 142.1 | 262.1 | 300.8 | 277.6 | 280.2 |
| $Zn_1$ | 157.3 | 145.7 | 158.4 | 153.8 | 263.3 | 252.3 | 293.8 | 269.8 |
| $Zn_2$ | 154.3 | 150.8 | 152.7 | 152.6 | 274.3 | 268 | 279.1 | 273.8 |
| $Zn_3$ | 155.1 | 157 | 166.3 | 159.5 | 270 | 269.7 | 291 | 276.9 |
| Mean | 151.2 | 151.5 | 153.2 | | 267.4 | 272.7 | 285.4 | |
| LSD $_{0.05}$ | N: ns Zn: ns NZn: 32.2 | | | | N: ns Zn: ns NZn: ns | | | |
| | $N_1$ | $N_2$ | $N_3$ | Mean | $N_1$ | $N_2$ | $N_3$ | Mean |
| | **Protein content in pods (g kg$^{-1}$)** | | | | **Carbohydrate content in pods (g kg$^{-1}$)** | | | |
| | **Second season (2016)** | | | | | | | |
| $Zn_0$ | 149.9 | 137.1 | 169.7 | 152.2 | 273 | 258.3 | 275.7 | 269 |
| $Zn_1$ | 141.2 | 164.3 | 152.2 | 152.6 | 265.3 | 257 | 278.7 | 267 |
| $Zn_2$ | 155.7 | 145.7 | 165.2 | 155.5 | 256.5 | 246.8 | 281.5 | 261.6 |
| $Zn_3$ | 152.8 | 167.3 | 175.3 | 165.2 | 277.2 | 296.3 | 277.3 | 283.6 |
| Mean | 149.9 | 153.6 | 165.6 | | 268.0 | 264.6 | 278.3 | |
| LSD $_{0.05}$ | N: 7.9 Zn: ns NZn: 21.4 | | | | N: 14.6 Zn: ns NZn:30.9 | | | |

*Notes: see footnote of Table 3 for treatment designation*

## Uptake of Zn, N, P and K by plant pods (Figures 1 to 4)

Zinc uptake (Figure 1) increased considerably by application of Zn. The no-Zn treatments exhibited between 32.4 to 35.2 g Zn ha$^{-1}$ whereas the Zn-treatments showed uptakes between 47.9 to 78.7 Zn ha$^{-1}$ indicating increases of up124% (when comparing with no-Zn treatments). The pattern of response to the Zn sources shows that the nano-Zn was the highest followed by the DTPA-Zn and that the sulphate Zn was the lowest among the three sources. This is a manifestation of the high efficiency of the nano-ZnO which enters into plant tissues via foliar spray (Lv *et al*., 2015).

Nitrogen uptake (Figure 2) was grater where Zn was sprayed. The increase in N uptake in pods due to foliar spray occurred in nearly all treatments sprayed with Zn. The nano-Zn and the DTPA-Zn were rather similar in effect except under conditions of the $N_2$ treatment where 72 kg mineral N + 72 kg organic N ha$^{-1}$. The increase in N uptake ranged from 6.7% caused by the Zn-sulphate under conditions of $N_2$ to as high as 64.3% caused by nano-ZnO under conditions of $N_2$.

Enhancement of protein in plant caused by nano-ZnO was observed by Kisan *et al*. (2015) in Spinach. Phosphorus uptake (Figure 3) increased by application of foliar spray. The increase caused by the nano-Zn under conditions of the $N_2$ treatment was highest. Under the fertilization with the $N_1$ and $N_3$ treatments, highest increase in P uptake in pods occurred with the Zn-DTPA.

Increases in P uptake ranged from 57.8% by the treatment receiving sulphate-Zn +144 kg N as mineral to as high as 140% by the treatment receiving DTPA-Zn + mineral N. Enhanced mobilization and release of P was reported by Raliya and Tarafdar (2013) upon application of nano-Zn to plants . Favourable effect of nano-Zn application was attributed by Lv *et al*. (2015) to creation of active Zn phosphate in plant, and that most applied nano-Zn converts to Zn-phosphates. Potassium uptake (Figure 4) increased due to application of foliar Zn. The highest increase was by applying 144 kg N kg or 72 kg mineral N + 72 kg organic N $ha^{-1}$ + biofertilization with DTPA-Zn foliar spray. In a study on peanuts, foliar spray with nano-Zn oxide surpassed foliar spray with Zn-sulphate by 30% in pod yield (Prasad *et al*., 2012). Increased yields of rice, sunflower and cowpeas were reported upon spraying the crops with nano-ZnO (Raddy, 2014).

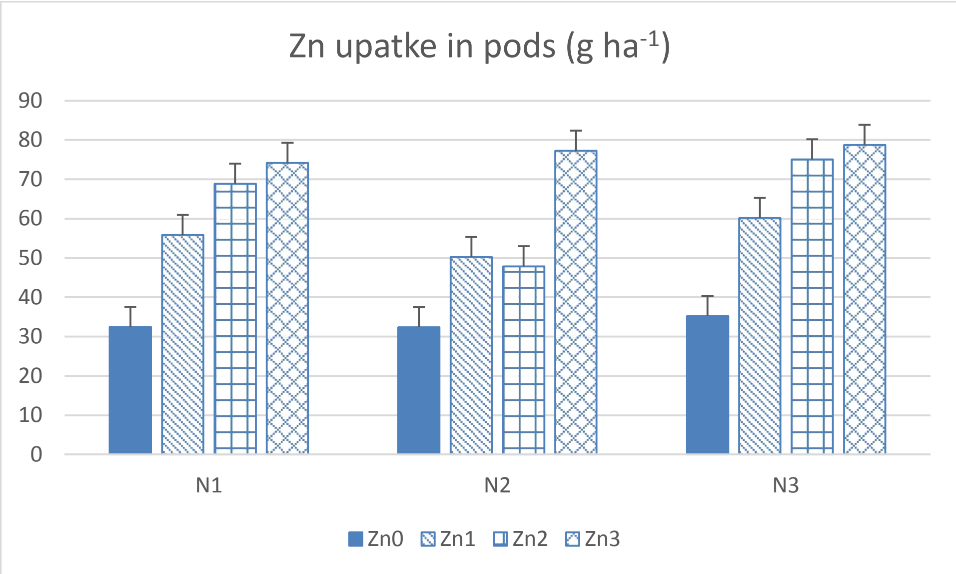


**Fig. 1:** Effect of Zinc foliar spray on snap beans using nano-Zn with N soil application of mineral, organic and biofertilizer: Zn uptake by plants (means of two seasons).

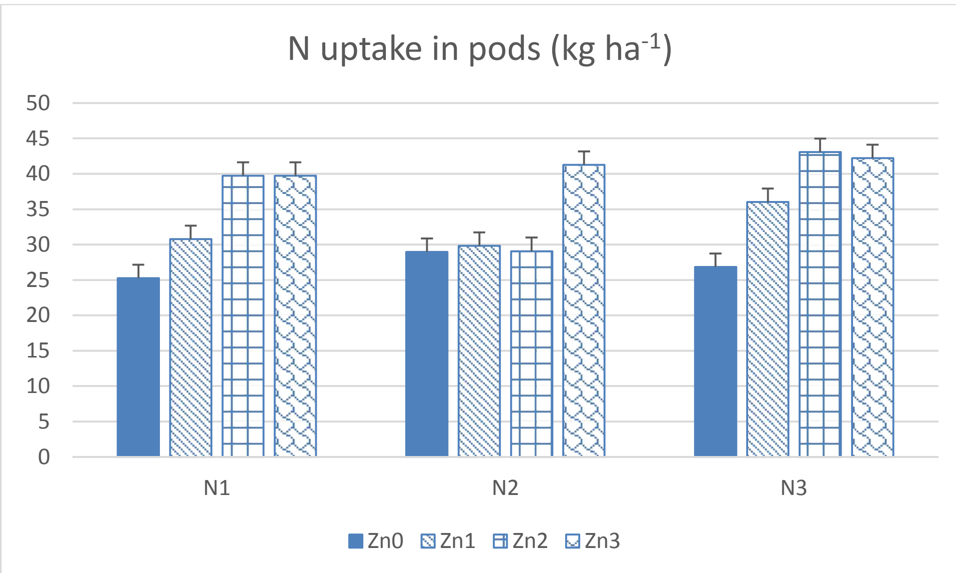


**Fig. 2:** Effect of Zinc foliar spray on snap beans using nano-Zn with N soil application of mineral, organic and biofertilizer: N uptake by plants (means of two seasons).

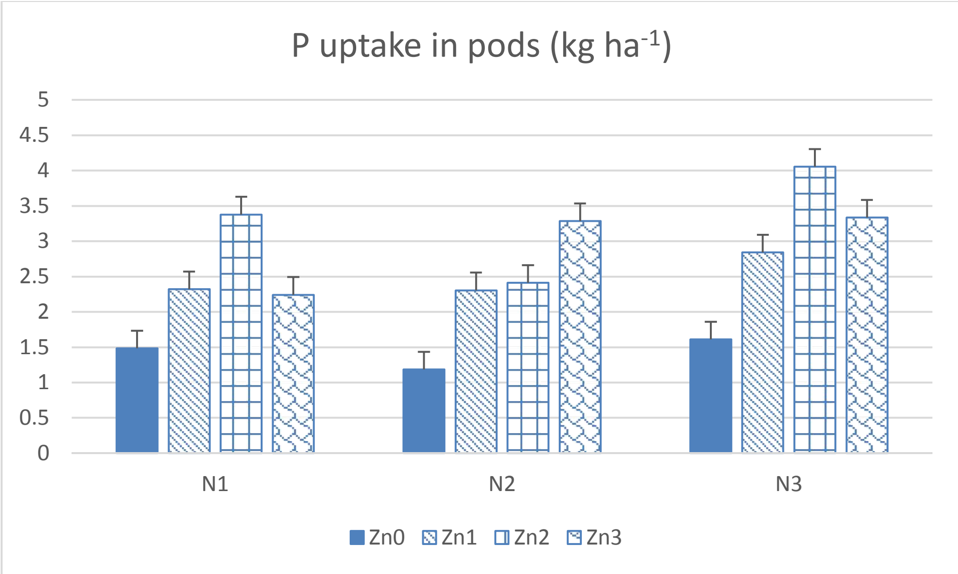


**Fig. 3:** Effect of Zinc foliar spray on snap beans using nano-Zn with N soil application of mineral, organic and biofertilizer: P uptake by plants (means of two seasons).

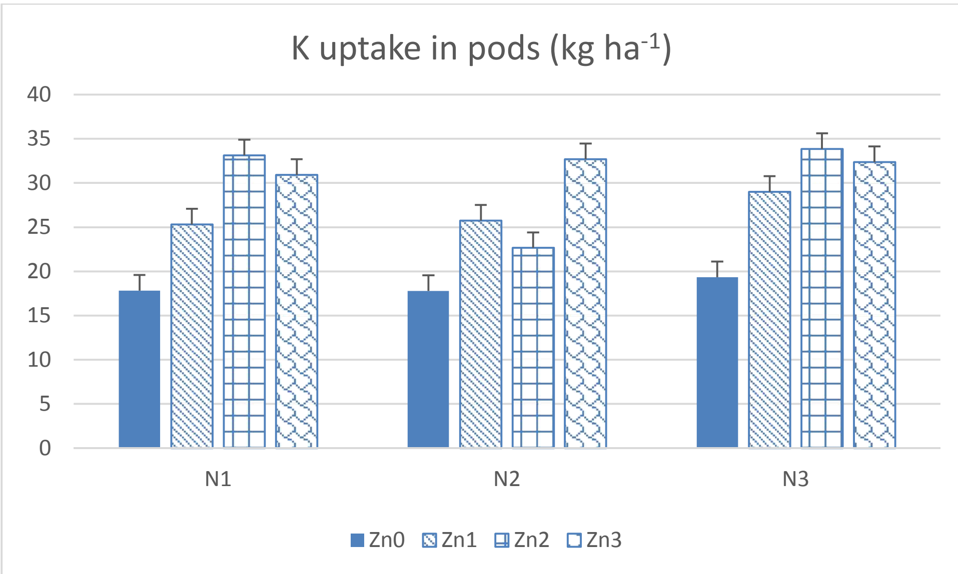


**Fig. 4:** Effect of Zinc foliar spray on snap beans using nano-Zn with N soil application of mineral, organic and biofertilizer: K uptake by plants (means of two seasons).

The positive effect of $N_3$ treatment on nutrient uptake could be attributed to biofertilizer (*Rhizobium phaseoli)* producing IAA, Indole Acetic Acid, (Arora *et al.,* 2001) thus increasing the root length with the eventual increase in acquiring nutrients from the soil (Gopalakrishnan *et al.,* 2015).

## Conclusion

Foliar-spraying of Zn in forms of sulphate, chelate, and nano-ZnO to snap bean (*Phaseolus vulgaris* L.) was effective in increasing yield as well as improving traits and quality of the crop and its pod production. The application of N in forms of mineral in combination of organic along with biofertilization with $N_2$-fixing rhizobium proved a very highly effective management in fertilization of the crop. Foliar spray wing non-ZnO was the most effective technology in foliar application of Zn micronutrient.

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