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Estimation of nitrogen use efficiency by mango seedlings under nano and convention calcium fertilization using the enriched stable isotope (N-15)

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$(^{15}NH_4)_2SO_4$

Nitrogen use efficiency

^{15}N uptake

ABSTRACT

This study aimed to investigate the effect of nano-Ca fertilizer on nitrogen uptake, nitrogen use efficiency and determine the best calcium form and dose for mango. A pot experiment was conducted using two year old mango seedlings (cv. Zebda). The pots were filled with sandy soil (8 kg per pot) and one seedling was transplanted into each pot. Four treatments including nano-Ca, convention Ca, soil application and foliar application have been formulated. Calcium was applied as CaO for both the convention and nanoforms. The enriched $(^{15}NH_4)_2SO_4$ was applied at a rate of (5g per pot). Plants were harvested at the end of the fall, spring, and summer growth cycles and dried at 70 ^{o}C. The dried plant is used for making fine powder and to determine total nitrogen, calcium, and $\%^{15}N$ atom excess. Results of the study revealed that in all growth cycles, the ^{15}N translocation was higher under foliar nano-Ca treatment than under convention Ca at a 100% rate. The highest uptake, translocation, and nitrogen use efficiency were observed at 50% (250 mg. L^{-1}) foliar nano-Ca treatment in all cycles. In the Fall growth cycle, the values for nitrogen fertilizer use efficiency at 50% nano-Ca rate was 81.8%, while it recorded 64.9% for 25% rate and 51.2% for 100% rate. Calcium concentration, in shoot and roots, was also higher under nano-calcium (for fall cycle = 3.0 for the shoot and 2.8 for root) than the convention calcium (for fall cycle = 2.7% for the shoot and 2.2 for root) for all cycles. The summer growth cycle recorded the highest total biomass under all treatments compared with the fall or spring growth cycles. Allocation of biomass to the shoot was also reported higher under nano-Ca foliar application than that of soil application in all cycles. The best treatment is 50% (250 mg.L^{-1}) foliar nano-Ca as it resulted in the highest N-15 uptake, translocation, and nitrogen use efficiency. Nano calcium proves to be more efficient as fertilizer than conventional calcium.

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1 Introduction

The current practices of fertilizer usage have caused serious environmental pollution, soil degradation, groundwater contamination, and economic loss. Fertilizers are not used efficiently and most of it is lost to the environment. Most conventional fertilizers have low nutrient supply efficiency (Elemike et al. 2019**)**. Around 40-70% of the nitrogen and 80-90% of the phosphorus fertilizers applied to the soil are lost to the environment or become unavailable to plants (Rajonee et al. 2017). To overcome the fertilizer loss, the nutrient deficiency of most soils, and the high cost of conventional fertilizer, the reduction in the use of chemical fertilizers is the recent approach to solve these problems. Instead, an increase in fertilizer use efficiency to obtain more yield per unit of fertilizer applied is needed.

Nanotechnology, serve as the latest technology which can provide less harmful and more efficient nano-fertilizer. Several researchers have reported that plant nutrients could be enriched by applying nano-nutrients that can be easily absorbed by plants. The use of nano-fertilizer is expected to help in reducing the fertilizer dose and increase plant productivity. Due to the small particle size (< 100 nm) and high surface area of nano-fertilizer it can easily penetrate the plant and improve uptake and nutrient use efficiency (Singh et al. 2017). Nanoscale (< 100nm) fertilizer has several distinguishing properties like a high surface area to volume ratio, acting as a slow-release fertilizer to provide nutrients throughout the crop growth period, reducing pollution of the environment (Corradini et al. 2010; Wilson and Rowe 2008). The beneficial effect of nanoparticles such as nano-TiO_2 increase photosynthetic rate, dry weight, chlorophyll a (Zheng et al. 2005; Khot et al. 2012); multi-walled carbon nanotubes (MWCNTs) increase the germination rate of tomato seeds by increasing the seed water uptake (Khodakovskaya et al. 2009) and nano-fertilizer prevent the plant from different biotic and abiotic stress (Singh et al. 2017). In addition, nano-scale particles with a higher surface area have a greater number of reaction sites than a particle with a low surface area, which results in enhanced chemical reactivity (Kale and Gawade 2016).

Mango (*Mangifera indica* Linn.) is one of the most important fruit crops in tropical and subtropical areas. It is considered to be the most popular fruit crop in Egypt. Growth of mango occurs in flushes of shoots from apical or lateral buds (Shaban 2009). Vegetative growth of mango trees occurs in both spring and summer cycles. The summer growth cycle recorded the highest significant vegetative growth, highest flowering percentage, the highest number of fruits per panicle, and fruit weight followed by the spring than the fall cycle (Shaban 2009).

Calcium is an essential component of cells, maintaining the structure of cell walls and stabilizing cell membranes (Abd El-Razek et al. 2017). Calcium levels exert a profound effect on the protein and soluble nitrogen content in different parts of Peanut and Linseed plants (Pal and Laloraya 1973). These researchers suggested that the level of soluble-nitrogen content is generally less in high calcium plants compared to the control.

The ^{15}N tracer technique is an isotope of broad application for the understanding of biological and/or chemical processes affecting nitrogen movement in the agriculture system (Axmann and Zapata 1990). A common form of fertilizer use efficiency expression is plant recovery or percent utilization of the added fertilizer (Zapata, 1990). Studying the uptake, translocation, and utilization of labeled nitrogen (N-15) can help optimize the dose of nano-calcium fertilizer for mango. The objective of this study was to investigate the effect of nano and convention Ca fertilizer, applied to the soil or as a foliar spray, on nitrogen uptake, translocation, and N recovery (nitrogen use efficiency) in the three growth cycles (Fall, Spring, and Summer) of mango seedlings. This will help to determine the best calcium form, dose, and method of Ca application for mango.

2 Materials and Methods

A pot experiment was conducted using two year old mango seedlings (cv. Zebda). The pots were filled with 8 kg per pot of sandy soil *(Typic torripsamments)* as per the recommendation of the Soil Survey Staff (1975). Plants were transplanted into the pots, one plant per pot. Each growth cycle contained 18 plants (6 treatments x 3 replicates).

2.1 N-15 labeled fertilizer

Nitrogen-15 fertilizer was applied as $(^{15}NH_4)_2SO_4$, enriched at 10.35% a.e. at a rate of 5g per pot after seedling transplanting. The labeled ammonium sulfate was manufactured at the Shanghai Research Institute of Chemical Industry, Shanghai, China.

2.2 Preparation of nano-calcium fertilizer

Nano fertilizers were prepared at the Soil Science Department, Faculty of Agriculture, Cairo University by ball-milling machine (Photon Company, Egypt). Portions of 100g of Ca fertilizer were placed in a stainless steel canister with metal balls of different sizes. The canister was placed on the ball-milling machine and stirred for 26 hours at speed of 1000 rpm/minute. A sample from the milled fertilizer was collected and submitted to the Transmission Electron Microscope (JEM-1400 TEM, Japan) at the Central Laboratories, Faculty of Agriculture, Cairo University for examination of the particle morphology and measuring the size of the Ca-nano particles. A drop of well-dispersed nanoparticles was placed onto the amorphous carbon-coated 200 mesh carbon grid, followed by drying the sample at ambient temperature, before loading it into the microscope (Wang et al. 2014). The size and morphology of the nanoparticles were measured and found to be less than 100 nm (Figure 1).

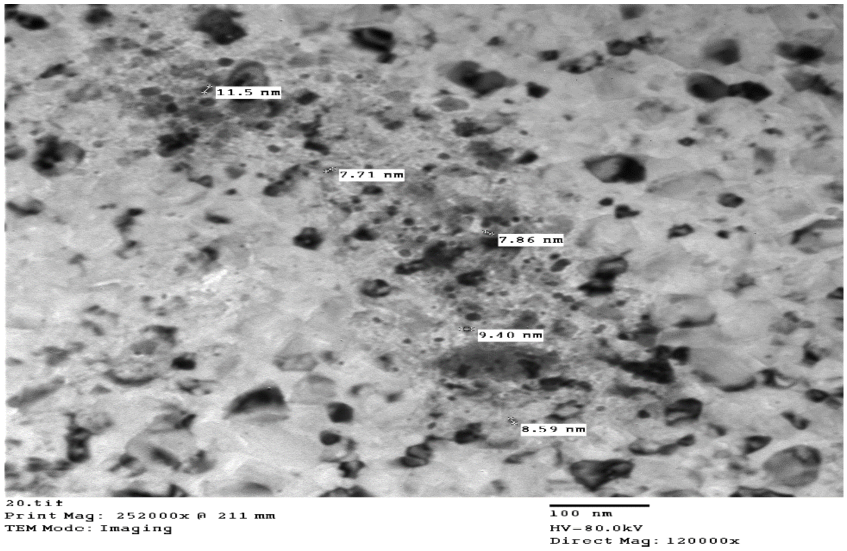

Figure 1 Transmission Electron Microscope Image of Nano-Calcium Particles

2.3 Treatments

Calcium fertilizer was applied as calcium oxide (CaO) for both the nano and convention forms, the following treatment were imposed for the recommended dose (i) Ca convention application in soil (100%=6g /pot), (ii) Ca convention application at foliar (100%= 500 mg.L^{-1}), (iii) Ca Nano application in soil (100%= 6g /pot), (iv) Ca Nano application at foliar (100% = 500 mg.L^{-1}), (v) Ca Nano application at foliar (50% = 250 mg.L^{-1}), and (vi) Ca Nano application at foliar (25% = 125 mg.L^{-1}).

2.4 Nutrient solution

Half strength modified Hoagland solution was prepared according to Stout et al. (1957). Half a liter of the prepared nutrient solution was added to each pot once a week. Plant watering was conducted to keep the soil moisture content at field capacity.

2.5 Plant harvest

The labeled ammonium sulfate fertilizer was applied at the beginning of the fall cycle in September. Plants of each growth cycle were harvested at different times. Plants of the fall growth cycle were harvested in December (after 3 months from the application of N-15), in May of the next year for spring, and in September for the summer growth cycle. Plants were washed with deionized water and separated into shoots and roots. All samples were dried at 70 ^{o}C and then ground up to very fine powder.

2.6 Nutrients analysis

Following plants harvest, they were analyzed for total nitrogen using a C/N analyzer (Elementar, Germany) and for % N-15 atom excess using the Emission Spectrometry N-15 Analyzer (FAN Fisher No.1-6PC Spectrometer). Calcium was measured using the Atomic Absorption Spectrometry (Shimadzu**6800**, Japan).

2.6.1 Calculation

The following equations were used for the calculation of the following parameters (Zapata, 1990):

Percent nitrogen derived from fertilizer (%Ndff) in plant organs

$$\%\text{N} - 15\ \text{dff} = \frac{\%\text{N a.e. in plant sample}}{\%\text{N a.e. in the labeled fertilizer}} \text{X } 100$$

Percent %^{15}N recovery by plant

$$\%\ \text{N} - 15\ \text{recovery (Utilization)} = \frac{\text{amount of N} - 15 \text{ in plant deried from fertilizer}}{\text{amount of N} - 15 \text{ applied to the soil}} \text{X } 100$$

2.7 Experiment Design and Statistical Analysis

Eighteen pots for each cycle were arranged in a Completely Randomized Design with three replicates for each treatment. Data were subjected to analysis of variance (Steel and Torrie 1960) using the MSTAT statistical microcomputer program. The significant means were compared using LSD at 5% probability (Snedecor and Cochran 1980). The results are an average of two seasons.

3 Results and Discussion

3.1 Nitrogen (^{15}N) uptake and translocation under Ca- application

Results in Tables (1, 2, 3) demonstrate that N-15 uptake under convention and nano-calcium applied to the soil at a 100% rate, differs in the different growth cycles. In the fall growth cycle, N-

15 uptake was significantly higher under convention Ca (%^{15}Ndff = 35.9) as compared to the nano- Ca (%^{15}Ndff =30.0). The opposite trend was observed in the spring growth cycle. The N-15 uptake was significantly higher under nano-Ca (% ^{15}Ndff =20.4) than under convention Ca (% ^{15}Ndff = 15.2). While in the summer growth cycle, the uptake was similar under convention Ca (%^{15}Ndff = 14.9) and nano-Ca ((%^{15}Ndff =14.8). Regarding N-15 translocation to the shoot, results show that the N-15 translocation to the shoot is significantly higher under nano-Ca than under convention-Ca fertilization in all cycles.

Comparing the N-15 uptake under foliar application of nano and convention Ca, at a 100% rate, it was higher under convention Ca (%^{15}Ndff = 34.8) than nano-Ca (%^{15}Ndff = 17.7) for the fall growth cycle. While in the spring cycle, the opposite trend was observed and the N-15 uptake was higher under nano-Ca by1.9 fold than the convention-Ca. In the summer growth cycle, the N-15 uptake was higher under nano-Ca by (1.5 fold) than the convention-Ca fertilization. The N-15 translocation in the fall and summer growth cycles was significantly higher under nano-Ca than convention-Ca by 1.6 and 1.5 fold, respectively. While the translocation in the spring was higher under nano-Ca as compared to the convention Ca but this difference was significantly not different. These findings agree with Swietlik (2006) who found that calcium enhanced the translocation of nitrogen.

3.2 Effect of foliar nano-Ca fertilization

The N-15 uptake and translocation, during the three growth cycles, at the different foliar nano-Ca application rates (100%, 50%, 25%) were compared. Results of the study revealed that N-15 uptake and translocation were highest at 50% nano-Ca foliar spray relative to 100% or 25% rates, in all three cycles. The trends of the N-15 translocation to the shoot are 50% > 100% >25%. Quantitative results of N-15 uptake and translocation showed that N-15 uptake for the summer growth cycle was 23.5 at 50%, while this was reported 22.7 and 19.6 for the 100% and 25% Ndff respectively. Results of the study also suggest that as Ca concentration increased N-15 uptake and translocation also increased. These results are in agreement with Swietlik (2006) who found that calcium enhanced the translocation of nitrogen.

3.3 Total nitrogen percent

Foliar application of nano-Ca in the fall and summer growth cycles at a 100% rate, showed significantly higher nitrogen content than soil application. Fall foliar application results are 1.20% for root and 1.42% for the shoot, while soil application results are 1.16% for root and 1.26 for the shoot. The highest total nitrogen percent was observed in the shoot and received a 50% foliar nano-Ca rate relative to 100% or 25% rates. Quantitative results showed that total nitrogen content in the shoot is 1.35% for the fall, while it was reported as 1.43% for the spring and 1.59% for the summer growth cycles. The percent N was significantly higher in the shoot than the root under both nano and convention Ca fertilization for both soil and foliar application in all cycles. The results agree with Swietlik (2006) who found that the application of calcium increased concentrations of N in the stem and roots of apple seedlings (*Malus x Domestica borkh.*).

3.4 ^{15}N recovery (nitrogen fertilizer use efficiency)

Results presented in Table 1 showed that percent N recovery in the fall growth cycle was significantly higher under nano-Ca (46.3%) than under convention Ca (30.7%) applied to the soil at 100% dose. Whereas, foliar application of Ca at 100% rate

Table 1 ^{15}N uptake, translocation, and recovery in the Fall growth cycle

Treatments	Method of application	Organ	%Ndff	%N	%^{15}N recovery	Amount of ^{15}N recovered
Convention 100%	Soil	Root	35.9	1.29	30.7	1.54
		Shoot	15.8	1.21		
Convention 100%	Foliar	Root	34.8	1.30	49.3	2.56
		Shoot	28.0	1.22		
Nano-Ca 100%	Soil	Root	30.0	1.16	46.3	2.32
		Shoot	38.3	1.26		
Nano-Ca 100%	Foliar	Root	17.7	1.20	51.2	2.47
		Shoot	44.8	1.42		
Nano-Ca 50%	Foliar	Root	33.9	1.23	81.8	4.09
		Shoot	45.5	1.35		
Nano-Ca 25%	Foliar	Root	20.0	1.11	64.9	3.25
		Shoot	39.9	1.30		
LSD (0.05)			1.91	0.02	2.10	0.08

showed higher %N recovery under nano-Ca (51.2%) than under convention Ca (49.3%), however, this difference is non-significant. The different concentrations of foliar nano-Ca showed that the highest % ^{15}N recovery was observed at nano-Ca concentration of 50% (81.8%) followed by 25% (64.9%), then 100% (51.2%). Nano-Ca at 50% treatment showed the highest ^{15}N-recovery and also the highest amount of ^{15}N recovered (4.09 g) among all treatments.

Results presented in Table 2 showed that %N recovery in the spring growth cycle was significantly higher under nano-Ca (75.4%) than under convention Ca (43.5%) applied to the soil at 100%. Foliar application of Ca at a 100% rate showed significantly higher %N recovery under nano-Ca (68.0%) than under convention Ca (59.2%). The different concentrations of foliar nano-Ca showed that the highest % ^{15}N recovery was observed at nano-Ca concentration of 50% (80.1%) followed by 25% (75.9%), then 100% (68.0%). Nano-Ca at 50% treatment showed the highest ^{15}N-recovery and the highest amount of ^{15}N recovered (4.01 g) among all treatments.

Results presented in Table 3 showed that %N recovery in the summer growth cycle was significantly higher under nano-Ca (63.6%) than under convention Ca (39.9%) applied to the soil at 100%. Foliar application of Ca at a 100% rate showed significantly higher %N recovery under nano-Ca (61.0%) than under convention Ca (49.3%). The different concentrations of foliar nano-Ca showed that the highest percent ^{15}N recovery was observed at nano-Ca concentration of 50% (90.4%) followed by 25% (70.4%), then 100% (61.0%). Nano-Ca at 50% treatment showed the highest ^{15}N-recovery and the highest amount of ^{15}N recovered (4.52 g) among all treatments.

Table 2 ^{15}N uptake, translocation and recovery in the Spring growth cycle

Treatments	Method of application	Organ	%Ndff	%N	%^{15}N recovery/plant	Amount of ^{15}N recovered
Convention 100%	Soil	Root	15.2	1.26	43.5	2.18
		Shoot	33.8	1.37		
Convention 100%	Foliar	Root	11.7	1.24	59.2	2.96
		Shoot	36.0	1.46		
Nano-Ca 100%	Soil	Root	20.4	1.29	75.4	3.77
		Shoot	36.0	1.49		
Nano-Ca 100%	Foliar	Root	22.2	1.24	68.0	3.40
		Shoot	38.0	1.43		
Nano-Ca 50%	Foliar	Root	25.8	1.21	80.1	4.01
		Shoot	38.8	1.43		
Nano-Ca 25%	Foliar	Root	19.5	1.19	75.9	3.80
		Shoot	37.3	1.42		
LSD (0.05)			2.46	0.02	1.09	0.09

Table 3 ^{15}N uptake, translocation, and recovery in the summer growth cycle

Treatments	Method of application	Organ	%Ndff	%N	%^{15}N recovery	Amount of ^{15}N recovered
Convention 100%	Soil	Root	14.9	1.16	39.9	2.00
		Shoot	22.0	1.31		
Convention 100%	Foliar	Root	15.3	1.34	49.3	2.47
		Shoot	20.8	1.50		
Nano-Ca 100%	Soil	Root	14.8	1.22	63.6	3.18
		Shoot	25.7	1.52		
Nano-Ca 100%	Foliar	Root	22.7	1.37	61.0	3.05
		Shoot	30.4	1.48		
Nano-Ca 50%	Foliar	Root	23.5	1.35	90.4	4.52
		Shoot	32.7	1.59		
Nano-Ca 25%	Foliar	Root	19.6	1.20	70.4	3.52
		Shoot	27.7	1.44		
LSD (0.05)			1.20	0.03	1.27	0.11

Results presented in tables (1, 2, 3 & 4) showed that nitrogen fertilizer use efficiency (^{15}N recovery), and N-15 translocation to the shoot were higher under nano-Ca than convention Ca for both application methods (soil and foliar) in all cycles. This is evidence of a synergistic relationship between nano-Ca and nitrogen which suggest that nano-Ca facilitate nitrogen translocation. Various previous researchers like Kale and Gawade, (2016) and Singh et al., (2017) have stated that nano fertilizer improves uptake and nutrient use efficiency due to its small size and high surface area.

3.5 Calcium concentration

Under soil calcium application, the root exhibited higher Ca concentration than shoot for both nano and convention calcium fertilization. On the contrary, shoot exhibited higher calcium concentrations than root under foliar calcium application for both nano and convention calcium. In general, nano calcium fertilization showed higher Ca concentration in the shoot and root tissues of mango plants than convention calcium for both application methods (soil and foliar) in all cycles. This could be due to the higher uptake and penetration of nano-Ca into the plant tissue. Our results agree with Singh et al. (2017) who stated that due to the small particle size (< 100 nm) and high surface area of nano-fertilizer it can easily penetrate the plant and improve uptake and nutrient use efficiency. Results in Table 4 show that calcium concentration in the leaf is in the sufficient range (2.0-3.4%) according to Jones et al. (1991). They mention that the sufficient range for Ca in mango leaf is (2.0-5.0%). The highest calcium concentration was observed at a nano-Ca concentration of 50% in all cycles.

3.6 Calcium-nitrogen interaction

Nano-calcium concentrations exert a profound effect on nitrogen content in the mango plant's shoot and root. Results show that nano-calcium foliar spray has increased nitrogen uptake, translocation, and nitrogen tissue concentration. In the fall growth cycle, the high level of foliar nano-Ca (100%) resulted in significantly higher root nitrogen content (%N=1.20%) than the low level (25%) (%N=1.11%). A similar trend was observed in the spring and the summer growth cycles. This is evidence of a synergistic relationship (positive interaction) between nano-calcium and nitrogen.

The calcium-nitrogen interaction suggested that our results agree with Gunes et al. (1998) who found that applied Ca increased the concentration of Ca and N in tomato plants. These results are also supported by Swietlik (2006) findings related to the calcium stimulation of nitrogen (in the ammonium form) uptake in apple and sour orange.

3.7 Total Biomass

Results in Table 5 showed that the plant total biomass is higher in the summer growth cycle followed by the spring growth cycle than the fall cycle. The average biomass of all treatments is 86.2g for the summer growth cycle, 71.8 g for the spring growth cycle, and 61.6 g for the fall growth cycle. In general, nano-calcium fertilizer resulted in significantly higher plant biomass than convention calcium fertilizer. This is could be due to higher ^{15}N uptake, translocation and nitrogen use efficiency under nano-calcium than convention calcium fertilization. The results are in the same line

Table 4 Calcium concentration in root and shoot of the three cycles

Treatments	Method of application	Organ	% Ca Fall	% Ca Spring	% Ca Summer
Convention 100%	Soil	Root	2.9	2.7	2.1
		Shoot	2.2	2.0	1.8
Convention 100%	Foliar	Root	2.2	2.0	1.7
		Shoot	2.7	2.5	2.0
Nano-Ca 100%	Soil	Root	3.3	3.4	3.0
		Shoot	2.6	2.3	2.3
Nano-Ca 100%	Foliar	Root	2.8	2.6	2.5
		Shoot	3.0	3.0	2.8
Nano-Ca 50%	Foliar	Root	3.2	2.9	2.8
		Shoot	3.4	3.3	3.1
Nano-Ca 25%	Foliar	Root	2.6	2.5	2.3
		Shoot	2.9	2.9	2.4
LSD (0.05)			0.23	0.15	0.17

Table 5 Plant total biomass and percent shoot biomass relative to the total

Treatments	Method of application	Fall cycle		Spring cycle		Summer cycle	
		Total biomass (g)	% shoot biomass	Total biomass (g)	% shoot biomass	Total biomass (g)	% shoot biomass
Convention 100%	Soil	59.4	75.4	52.5	81.9	76.0	77.6
Convention 100%	Foliar	70.5	80.9	68.6	75.2	85.8	76.7
Nano-Ca 100%	Soil	51.6	75.0	81.1	73.7	90.1	81.9
Nano-Ca 100%	Foliar	44.7	80.1	68.0	83.8	71.2	84.6
Nano-Ca 50%	Foliar	72.0	76.7	80.0	77.5	94.5	79.4
Nano-Ca 25%	Foliar	71.5	78.0	80.6	80.1	99.7	71.9
LSD (0.05)		1.50	1.80	2.00	1.37	2.10	1.80

with Mahmood et al. (2009) who observed a significant highly positive correlation between dry matter yield and Ca concentration in wheat plant tissue.

Allocation of biomass to the shoot was higher under foliar application (80.9%) than the soil (75.4%) application method for convention calcium fertilization (100% rate) for the fall growth cycle, but lower for the spring growth cycle and almost similar for the summer growth cycle. Allocation of biomass to the shoot was higher under Ca foliar application than the soil application method for nano-calcium fertilization at a 100% rate for all growth cycles. Comparing the values of percent shoot biomass under nano and convention Ca-fertilizer, results revealed similar values (75.4%, 75.0%) for the fall cycle, while it recorded a higher value for the convention (81.9%) than nano-Ca (73.7%) for the spring cycle and higher value for nano-Ca (81.9%) than convention Ca (77.6%) for the summer growth cycle. Comparing the 3 levels of foliar nano-calcium, allocation of biomass to the shoot was significantly highest at 100% followed by 50% then 25% for all cycles. Results of the total biomass revealed that the summer growth cycle has the highest total biomass under all treatments compared with the fall or spring cycles. The summer growth cycle was reported to give the highest flowering percentage, number of fruits per panicle, and fruit weight followed by the spring growth cycle and then the fall growth cycle (Shaban 2009). The higher nitrogen use efficiency under nano than convention calcium fertilizer has resulted in higher plant biomass in the former than the latter. Similar results were obtained by Laware and Raskar (2014) who demonstrated that onion crops treated with ZnO-nano particles at 20 and 30 µg/ml showed better growth and flowered 12-14 days earlier than those treated with convention $ZnSO_4$.

The total biomass results presented in Table 5 showed that the summer growth cycle recorded the highest total biomass under all treatments compared with the fall or spring cycles. The summer growth cycle was reported to give the highest flowering percentage, the number of fruits per panicle, and fruit weight followed by the spring cycle then the fall cycles (Shaban 2009). The higher nitrogen use efficiency under nano than convention calcium fertilizer has resulted in higher plant biomass in the former as compared to the later. Similar results were obtained by Laware and Raskar (2014) who demonstrated that onion crops treated with ZnO-nano particles at 20 and 30 µg/ml showed better growth and flowered 12-14 days earlier than those treated with convention $ZnSO_4$.

Conclusions

In conclusion, nitrogen use efficiency (^{15}N recovery) was higher under nano-calcium than convention calcium fertilization in all cycles. This finding proves true under the two application methods (soil and foliar). There is evidence of a positive synergistic relationship between nano-Ca and nitrogen, i.e. nano-Ca enhanced ^{15}N uptake, translocation to the shoot, N tissue concentration, and ^{15}N recovery. The highest N-15 uptake and translocation was observed at a nano-Ca foliar concentration of 50% relative to 25%. A significantly higher %N was observed in the shoot than the root under nano-Ca fertilization for both soil and foliar application methods in all cycles. Calcium concentration in mango seedlings was higher under nano-calcium than under convention calcium fertilization in all cycles. The summer growth cycle recorded the highest total biomass compared with the fall or the spring growth cycles. Results of the current study recommend applying calcium in the nano form at 50% of the recommended rate as a foliar spray.

Conflict of interest

The authors declare no conflict of interest

References

Abd El-Razek E., Amin, O.A., El-Nahrawy, A.M., & Abdel-Hamid, N. (2017). Effect of foliar application of biosimulated nanomaterials (calcium/yeast nanocomposite) on yield and fruit quality of “Ewais” mango trees. *Annual Research & Review in Biology*, *18*, 1-11.

Axmann, H., & Zapata, F. (1990). Stable and radioactive isotopes. In Hardarson, G. (ed.) Uses of Nuclear Techniques in Studies of Soil-Plant Relationships, (pp. 9-34) Training course Series No. 2, IAEA, Vienna-Austria.

Corradini E., de Moura, M. R., & Mattoso, L. H. C. (2010). A preliminary study of the incorporation of NPK fertilizer into chitosan nanoparticles. *EXPRESS Polymer Letters*, *4,* 509–515.

Elemike E.E., Uzoh, I.M., Onwudiwe, D.C., & Babalola, O.O. (2019). The role of Nanotechnology in the Fortification of plant nutrients and Improvement of Crop production. *Applied Sciences*, *9,* 1-32.

Gunes, A., Alpaslan, M., & Inal, A. (1998) Critical nutrient concentrations and antagonisticand synergistic relationships among the nutrients of NFT-grown young tomato plants. *Journal of Plant Nutrition*, *21,* 2035-2047.

Jones J.B., Wolf, B., & Mills, H.A. (1991). *Plant Analysis Handbook*. Micro-Macro Publishing, Inc., Athens-GA, USA. pp. 195-203.

Kale A.P., & Gawade, S.N. (2016). Studies on nanoparticle induced nutrient use efficiency of fertilizer and crop productivity. *Green Chemistry & Technology Letters*, *2*, 88-92.

Khodakovskaya, M., Dervishi, E., Mahmood, M., Xu, Y., Li, Z., Watanabe, F., & Biris, A.S. (2009). Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. *ACS Nano 3*, 3221–3227.

Khot L.A., Sankaran, S., Maja, J.M., Ehsani, R., Schuster, E.W. (2012). Applications of nanomaterials in agricultural production and crop protection: a review. *Crop Protection*, *35,* 64–70.

Laware, S.L., & Raskar, S. (2014). Influence of zinc oxide nanoparticles on growth, flowering and seed productivity in onion. *International Journal of Current Microbiology Applied Sciences*, *3,* 874-881.

Mahmood, I.A., Salim, M., Ali, A., Arshadullah, M., Zaman B., & Mir, A. (2009). Impact of calcium sulphate and calcium carbide on nitrogen use efficiency of wheat in normal and saline sodic soils. *Soil and Environment*, *28,* 29-37.

Pal, R.N., & Laloraya, M.M. (1973). Calcium in relation to nitrogen metabolism: I- Changes in protein and soluble-nitrogen in Peanut and Linseed plants. *Biochemie und Physiologie der Pflanzen*, *164,* 315-326.

Rajonee, A.A., Zaman, S., & Imam ulHuq, S.M. (2017). Preparation, characterization and evaluation of efficacy of phosphorus and potassium incorporated nano fertilizer. *Advances in Nanoparticles*, *6,* 62-74.

Shaban, A.E.A. (2009). Vegetative growth cycles of some mango cultivars in relation to flowering and fruiting. *World Journal of Agricultural Sciences*, *5,* 751-759.

Singh, M.D., Chirag, G., Prakash, P.O., Mohan, M.H., Prakasha, G., & Vishwajith (2017). Nanofertilizer is a new way to increase nutrients use efficiency in crop production. *International Journal of Agriculture Sciences*, *9,* 3831-3833.

Snedecor, D.W., & Cochran, W.G. (1980). Statistical methods. 7th Ed. Iow State University Press, Ames-Iowa, USA.

Soil Survey Staff (1975). Soil Taxonomy. Agric Handbook, No. 436, USDA, Washington D.C.

Steel, R.G.D., & Torrie, J.H. (1960). *Principles and Procedures of Statistics*. McGraw-Hill Book Company Inc., New York, USA.

Stout P.R., Broyer, T.C., & Carlton, A.B. (1957). Comparative chlorine requirements of different plant species. *Plant and Soil*, *8,* 337-353.

Swietlik, D. (2006). The interaction between calcium chloride and ammonium-nitrogen on growth, nitrogen uptake and translocation in apple and sour orange. *Acta Horticulturae*, *721,* 159-164.

Wang, C., Luo, H., Zhang, Z., Wu, Y., Zhang, J., & Chen, S. (2014). Removal of As (III) and As (V) from aqueous solutions using nano scale zero valent iron-reduced graphite oxide modified composites. *Journal of Hazardous Materials*, *268,* 124-131.

Wilson, P. G., & Rowe, R. (2008). A revision of the Indigofereae (Fabaceae) in Australia. 2: Indigofera species with trifoliolate and alternately pinnate leaves. *Telopea, 12,* 293–307.

Zapata, F. (1990). Isotope techniques in soil fertility and plant nutrition studies. In: Hardarson, G. (ed.) *Use of Nuclear Techniques in Studies of Soil-Plant Relationships*, (pp. 61-128), Training course Series No. 2, IAEA, Vienna-Austria.

Zheng, L., Zheng, L., Zhou, M., Chai, Q. et al. (2005). Novel function of the flap endonuclease 1 complex in processing stalled DNA replication forks. *EMBO Reports*, *6,* 83-89.