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The Effect of Nitrogen Fertilizer and Mycorrhizal fungi on productivity of Citrus Trees Grown in Newly Reclaimed Soil

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ABSTRACT

This study was conducted during two consecutive seasons using the bio-stimulant Mycorrhizal fungi and/or three nitrogen forms applied to Valencia orange orchard (*C. sinensis* .L) grown in a private orchard at El-Tahreer region, Al- Behera Governorate, Egypt. The aim of this research was to study the effect of nitrogen forms and Mycorrhizal fungi on vegetative growth, leaf mineral content, yield and fruit quality of citrus orchards grown in newly reclaimed soil. Mycorrhizal fungi (VAM) was inoculated at two levels (10 or 20 ml. of spore suspension/tree) expressed as VAM I or II - each ml. contains 50 spores. Also, tested fertilizers were applied in nitrogen forms once at spring growth to obtain actual N at 1000g /tree resembling 2.150 Kg Urea (46.5% N) or 2.600 Kg Urea Formaldehyde (38.37% N) or 2.500 Kg Sulfur-Coated Urea (40% N).

Results recorded that sole application of nitrogen source as slow release fertilizer in SCU or UF form were favorable for improving VAM infection percentages as well as different tested parameters compared to the fast one, where SCU treatment dominated in this respect. Furthermore, Mycorrhizal trees at low or high level, equally recorded best values for vegetative growth, macro- and micro-nutrient contents besides its superiority in improving yield and fruit quality as fertilized by N in SCU form. Also, it is noticed that UF treatment arranged the second order as applied to Mycorrhizal trees, only at high level. In addition, high VAM level proved to be essential for enhancing Urea fertilizer to produce pronounced values of tested parameters. This means that application of Sulfur Coated Urea to VA Mycorrhizal trees at either level is highly recommended for Citrus orchards grown in calcareous soils to promote VAM infection, tree growth, minerals availability and consequently produce high yield of good quality.

However, application of SCU to low VA Mycorrhizal trees gave similar effect that alleviate the dependence on urea as costly commercial fertilizers due to its greater potential for N loss via ammonia volatilization.

Key word: Orange trees, VA Mycorrhizal fungi, slow release fertilizers, nitrogen, Fruit quality, Yield.

Introduction

Citrus is one of the important fruit trees in Egypt which occupy the first level, its acreage reached 333.090 Feddan whereas Sweet Oranges (*Citrus sinensis* L.) resembling the most extensively grown form. Due to the future wide exportation of Valencia orange, its cultivation area gradually increased to produce about 660,000 tons with average 10.13 ton/fedd. Where Egypt's top export destinations in 2012/2013 included European Union, Russia, Saudi Arabia, Ukraine, the United Arab Emirates and Iraq. Most of Valencia orange acreage located in newly reclaimed areas because of its tolerance to high temperature and low humidity. (Global Agric. Information Network, Egypt Citrus Annual Report 2013/2014).

Nitrogen is known to be essential nutrient for plant development because of its role in proteins, nucleic acids and enzymes synthesis (Kandil *et al.,* 2010 and Hassan *et al.,* 2010). Nitrogen applied as urea or NH_{+4} undergoes chemical transformation to produce either (NH_3) or (NO_{-3}) depending on soil pH, application methods and moisture conditions, where ammonia volatilization may contribute substantially to N loss in Calcareous sandy soils that cover a large proportion of newly reclaimed soils (He *et al.,* 1999). Furthermore, extensive studies were conducted to quantify N leaching loss in Calcareous sandy soils because of public concern that nitrate is not absorbed by tree root system can be leached below the root zone and results in contamination of the groundwater (USEPA, 1987; Mc Neal *et al.,* 1995 and Paramasivam *et al.,* 2001). Furthermore, Kithome *et al*. (1998) reported that ammonium retained in soil by subjecting to slow release through cation exchange and nitrification, where the efficiency of slow release nitrogen forms can potentially reduce nitrogen losses, improve the efficiency of plant recovery and decrease soil pH due to the acidic effect of urea-formaldehyde or sulfur coated urea. It is well known that lowing soil pH improves the availability of many nutrients needed for growing plants, (Diez *et al.,* 1994; Saleh and Abd El-Kader, 2004 and Ciprian and Iovu , 2010).

So, it is of important to improve the efficiency of nitrogen fertilizer by using other nitrogen forms, techniques and alternative systems. Out of those, the application of controlled release N fertilizers *i.e* Urea formaldehyde that developed mainly to reduce the replication additive number per year, minimize production cost, improve the efficiency of N used by trees, reducing N leaching and controlling nitrate pollution (Eman *et al.,* 2009 and Abdou A.

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Soaud *et al.,* 2011). Also, sulfur-coated urea (SCU) that contains 30-40 % N and 15-20% S, usually used to promote rhizosphere activity, reduce soil pH and subsequently increases the availability of soil nutrients (Boutros *et al.,* 1995).

Similarly, considerable attention has been attracted to the possibilities of using VA Mycorrhizae fungi as agro-technical factor for increasing nutrients uptake in fruit orchards. The principal function of these associations is enhancing the solubility of different nutrients and the efficiency of its absorption (Marschner,1995 and Bâ *et al.,* 2001).Trees treated with Mycorrhizal fungi accumulate more K, Ca, Cu and Mn in plant leaves (Miller *et al.,* 1985 and Fernando Borie *et al.,* 2010). VAM fungi interact with other soil microbes like free-living nitrogen fixers to improve their efficiency for the biochemical cycling of elements to host plants (Linderman, 1988). These microorganisms proved to be more effective in correcting nutrient deficiencies and maintaining proper metal balance in plant tissues (Nelson and Achar, 2001; Gupta *et al.,* 2002; Huiying Li, 2005 and Tabassum *et al.,* 2012). However, there is relatively limited information concerning performance of Valancia orange trees bio-fertilized with VAM Fungi under sandy Calcareous soil conditions.

Hence, these experiments were undertaken to evaluate the efficiency of nitrogen fertilizer as identified by other nitrogen forms and alternative systems *i.e,* the slow release N fertilizers namely urea formaldehyde, Sulfur coated urea and /or VA Mycorrhizal inoculations to reduce NH_3 volatilization and maximize nitrogen efficiency in Valancia orange orchards.

Materials and Methods

The current study was imposed during 2011 and 2012 seasons on 16 years old Valancia orange trees *(Citrus sinensis* L*)* budded on sour orange rootstock grown at 5x5 meters a part grown in a private orchard at El-Tahreer region, Al- Behera Governorate, Egypt. All trees were almost uniform in vigor, grown in a soil classified as sandy calcareous under drip irrigation system with conventionally accepted practices including fertilizing rate: 1000*g* N, 200*g* P_2O_5 and 600*g* K_2O per tree. Soil properties were determined according to (Wilde *et al.,* 1985) as shown in Table 1.

Table 1: Soil properties:

Constituents	Values %	Constituents	Values	Constituents	Values Meq L^{-1}	Constituents	Values Meq L^{-1}
Sand	80.3	*pH 1: 2.5 extract*	8.0	Exch. K+ Mg/100 g	19.6	Na	3.10
Silt	11.8	Total CaCO3%	7.9	Exch. Ca++ Mg/100 g	21.5	Cl	3.40
Clay	7.9	Available P Mg/100 g	3.25	Mg Meq L^{-1}	1.50	So_4	3.88

Two weeks after manure addition, trees were inoculated with VA Mycorrhizal fungi (VAM) spores at 2 levels (10 or 20 *ml*. of spore suspension/tree) expressed as VAM I or II - each *ml.* contains 50 spores. Spore suspension was prepared by wet- sieving and decanting procedure [24], diluted to reach 10 *ml*. and boarded throughout root growth area. Tested fertilizers were used as N source to obtain actual N at 1000*g* /tree, resembling 2.150 Kg Urea (46.5% N) or 2.600 Kg Urea formaldehyde (38.37% N) or 2.500 K*g* sulfur-coated Urea (40% N). Other horticultural practices were applied as usual.

Urea formaldehyde or sulfur-coated Urea were applied once as spring start, while the fast release one (Urea) was added twice at spring growth start and after fruit set. Each N form was also examined in trees inoculated with VAM levels that received the basal P and K fertilizers comparing with uninoculated one(control).. Treatments were arranged in complete randomized block design with three replicates each represented by two trees.

The effect of VAM inoculation levels and / or nitrogen fertilizer forms on infection percentages, growth parameters, physical and chemical characters of Valancia orange fruits, were determined as follows:

VAM infection %:

Root samples were digged, cleared, stained with lactophenol cotton blue and spores were counted as described by (Musandu and Giller,1994).

Vegetative parameters:

- Shoot length and number of shoots / branch:

Four branches uniform in diameter and length, distributed on tree directions were labeled at first of April. Number of developing shoots per branch were counted and tagged to determine shoot length as growth ceased at mid-December.

- *Leaf Area* :
Samples of 20 leaves (the third leaf from the base of the tagged non fruiting shoots) were collected in mid-September and leaf area was determined using Planimeter1800s.
- *Total chlorophyll contents***:**
Leaf chlorophyll were extracted with 100% Ethanol and the analysis of absorbance (649 & 668 *nm*) were made using Spectrophotometer. Chlorophyll concentrations were calculated and expressed on leaf dry mass basis according to Wintermans and Demots (1965).
- *Leaf Total Carbohydrates*:
Leaf Total Carbohydrates was determined according to Smith *et al.* (1986).
- *Leaf dry Weight***:**
Leaves were washed, oven dried till a constant weight, and recorded.
- *Relative water -content (RWC):*
Relative water -content (RWC) was determined using the following formula: RWC% = (FW - DW) / (SW - DW) x 100.

Where: FW = leaf fresh weight, DW= leaf dry weight.

Leaf chemical contents

Forty leaves were taken at late August from none fruiting and none flushing tag spring growth cycle. Samples were dried, grounded and digested by sulphoric acid and oxygen peroxide. Leaf mineral content of N, P, K, Ca, Mg, Zn, Fe and`Mn were determined on dry weight basis according to the method described by (Rebbeca, 2004).

Fruit physical characters:

Fruit weight (*g*), number, length (L) diameter (D) / *cm*, volume (*ml*.), peel thickness *(mm*) and Yield *kg/*tree were measured, where fruit shape index calculated as L/D ratio**.**

Fruit chemical contents

Edible rate was calculated as pulp / fruit weight %. Where in fruit juice, **S**oluble Solid Content % was determined using hand refractometer, total acidity % was determined as citric acid, and S.S.C/ acidity % was calculated, Vitamin C (*mg*/100g F.Wt.) was determined by titration with 2-6 Dichlorophenol – Indophenol and total sugar contents estimated according to AOAC, 1985.

Obtained data were subjected to analysis of variance according to the procedure reported by Snedecor, 1989. Means were compared by the least significant difference test (LSD) at 5% level of probability.

Results and Discussion

Infection percentages and growth parameters

The effects of VAM inoculation levels and / or nitrogen fertilizer form on infection percentages and growth parameters of Valancia orange trees grown in calcareous soil are presented in (Table 2).

VA Mycorrhizal infection%:

Data indicate that infection% of Mycorrhizal inoculation was positively increased with inoculation levels. Fertilization with the fast release Urea (U) reflected nearly double folds as added to trees with highest VAM inoculation rate, as compared with un-inoculated control. Also, slow release N form increased infection % to reach 42.8 or 42.2 with sulfur coated urea (SCU) as combined with either low or high inoculation level, respectively. Also, high infection percentage (37.7 %) was significantly obtained with high inoculation level in trees fertilized with Urea Formaldehyde (UF).

These results are in harmony with Fernando *et al*. (2010) who mentioned to the role played by AM symbiosis in such soils and provide guidance on the most appropriate alternatives to increase its presence and functionality. Also, Bâ *et al*. (2001) reported that Sulpher amendment increases root penetration depth, improve soil properties and possibly soil aeration, as well as promoting rhizosphere activity in sandy soil.

Growth parameters:

Shoot length, number of shoots / branch and leaf area were taken as indication of growth in tested trees as shown in (Table 2). Trees inoculated with VAM at high level significantly increased tree growth as treated with different N fertilizer forms compared to the un inoculated U control. Trees fertilized with SCU at either inoculation level, proved to be the superior treatments in enhancing shoot length (59.9 &60.2 *cm*.), number of shoots / branch (6.2&6.3) and leaf area (30.3&30.5 Cm^2) with similar positive effect , followed by trees inoculated with high VAM level and fertilized with U.F. form than those treated with low VAM level + U.F. fertilizer.

Leaf Total Chlorophyll Content:

Table (2) show that inoculated trees treated with tested N forms induced a pronounced positive effect on leaf total chlorophyll content as compared with un-inoculated U control. In this respect, Trees inoculated with either high or low VAM level and treated with S.C.U took the superiority in enhancing chlorophyll synthesis (5.5& 5.7*mg/L*) followed in descending order by trees treated with VAM at high level + U.F.(4.9 *mg /L*). Moreover, application of U either alone or combined with low VAM level, induced the lowest positive values followed in ascending order by U+ VAM at high level.

Leaf Dry Weight

Recorded data show that trees fertilized with either N form of slow release fertilizers produced higher leaf dry weight in comparison with the analogous ones fertilized with the traditional fast release nitrogen fertilizer "control". Moreover, treatments surpassed SCU as combined with both VAM levels in producing highest leaf dry weight values (56.5& 57.3 %), with non-significant differences. However, applying UF to trees inoculated with high VAM level revealed positive response (57.0 %), in this respect. Contrarily, least Dry Weight values were significantly obtained with U treatment, either as sole application or in combination with lower VAM level.

Table 2: Effect of VAM inoculation and / or N forms on infection percentages and some growth parameters of Valancia orange trees grown in calcareous soil (Mean of two seasons).

N form	VAM level	VAM infection %	No. of Shoots/ branch	Shoot length (*cm*)	Leaf			Carbo-hydrates %	Chloro-phyll (*mg/L*)
					Area Cm^2	D.Wt. %	RWC %		
U	0	14.1 f	5.5 c	46.6 f	24.8 e	47.9e	81.2a	16.9 gh	2.2 ef
	I	17.9 e	5.6 c	47.6 f	25.4 d	50.8d	80.8a	17.6 g	2.6 e
	II	29.2 c	5.9 b	51.0 d	27.9 c	51.6cd	80.3a	19.9 d	4.1 c
SCU	0	25.8 d	6.0 b	54.4 e	27.3 c	54.9b	77.5b	19.1 e	3.6 d
	I	42.2 a	6.2 a	59.9 a	30.3 a	56.5a	73.0c	22.1 a	5.5 a
	II	42.8 a	6.3 a	60.2 a	30.5 a	57.3a	73.9c	22.3 a	5.7 a
UF	0	22.2 e	6.0 b	57.9 e	27.0 cd	52.1c	78.2b	18.4 f	3.2 d
	I	33.9 b	6.0 b	61.8 c	29.5 b	54.6b	77.4b	20.7 c	4.5 bc
	II	37.7 ab	6.1 b	48.7 b	30.2 a	57.0a	73.2c	21.5 ab	4.9 b

**U: urea (46.5% N); U.F.: Urea formaldehyde (38.37% N) ; S.C.U.: Sulfur-coated urea. (40 % N)*

** Means in columns followed by the same letter are not significantly different Means according to L.S.D. test (P 0.05).*

Leaf Total Carbohydrates (%):

Tabulated data reveal that different N forms surpassed the traditional fast release nitrogen fertilizer "control", especially with trees inoculated by VAM at higher level. Generally, the higher values of total carbohydrates (22.3 &22.1%) were significantly obtained as SCU treatment combined with higher or lower inoculation level, respectively. Moreover, enhancing effect of UF induced high Carbohydrate values in trees inoculated with high VAM level (21.5%). Other treatments took an intermediate position in this concern.

The importance of nitrogen as essential constituent of chlorophyll, protoplasm, proteins and nucleic acid for increasing growth parameters, was discussed by Nijjar (1985); Wassel *et al.,* (2000) and Mario *et al.,* (2008). Where, the improving effect of SCU and UF as slow release fertilizers on vegetative growth might be attributed to their low activity index, that regulating the N release according to plant need, compared with fast release one (urea) which gave the lowest values of available N left in the soil (Mikkelesen *et al.,* 2001; and Kandil *et al.,* 2010). In addition, the dominance in growth parameters due to SCU treatment may be attributed to acidification resulted from Sulfur oxidation that decrease soil pH and enhance nutrients solubility besides increasing the activity of micro-organisms (El-Tarabily *et al.,* 2006 and Abdou A. Soaud *et al.,* 2011).

Concerning VAM effects, the increment in growth of inoculated trees may be due to the ability of *VAM* fungi to produce some growth promoting substances, organic, inorganic acids and CO_2 which lead to increase in soil acidity and consequently convert the insoluble forms of nutrients into soluble ones (Boutros *et al.,* 1995 ; Nelson and Achar, 2001 and Tabassum *et al.,* 2012). The VAM hyphae also help in retaining moisture around the root zone of plants (Bâ *et al.,* 2001 and Morte *et al.,* 2001) which reflect efficient mobilization and uptake of nutrients. Gupta *et al* (2002**)** and Tabassum *et al*. (2012) reported that VAM fungi interact with other soil microbes like free-living nitrogen fixers and phosphate solubilizers to improve their efficiency for the biochemical cycling of elements to the host plants. This may explain why the highest growth values were detected in trees inoculated with VAM fungi.

Leaf chemical contents

Table (3) demonstrates the effect of VAM inoculation and / or nitrogen forms on leaf macro and micro-nutrient contents of Valancia orange trees.

Macro nutrient contents%:

*Nitrogen**:*** The sole application of different N forms including control succeeded in enhancing leaf nitrogen content in the optimum range according to current guide lines. Applying either tested N form to inoculated trees induced statistically similar and higher positive effect. In this respect, SCU dominated in increasing nitrogen content (2.23 & 2.24 %) against (1.91 & 2.12%) for control treatment as combined with low and high level of VAM treatment, respectively. Moreover, UF + high VAM level exerted comparatively high enhancing effect on leaf nitrogen content (2.31 %).

Phosphorus: Tabulated data declare that all tested treatments enhanced leaf phosphorus content in comparison with the un inoculated control. Generally, inoculated trees at high level scored higher leaf phosphorus contents (0.225 & 0.211 and 0.192 %) for SCU, UF and U forms, respectively. In addition, P content was within the high range in trees treated with VAM at low level + SCU fertilizer.

*Potassium***:** Table (3) demonstrates that both slow release nitrogen fertilizers surpassed the fast release one for increasing leaf K values. Potassium contents for SCU treatment scored the highest significant values (1.92&1.93 %) as combined with VAM at low or high level, respectively. Moreover, combined effect of high VAM level + UF produced similar highest values (1.91%) with non significant differences.

Calcium: Obtained data illustrate that SCU + VAM treatments at either level recorded the highest leaf calcium values (1.63&1.61%). Similarly, UF +VAM treatments at either level scored the second order, in this respect (1.57 and 1.53 %). Furthermore, U treatment produced the lowest significant effects as applied alone(1.34 %) or combined with low VAM inoculation rate (1.32 %).

Table 3: Effect of VAM inoculation and / or nitrogen forms on leaf chemical contents of Valencia orange trees grown in calcareous soil. (Mean of two seasons).

N Form	VAM level	Macro nutrient contents %					Micro nutrient %		
		N	P	K	Ca	Mg	Fe	Zn	Mn
U	0	1.87 h	0.148h	1.55 g	1.34 e	0.256 e	38.3 h	23.9 g	21.5 g
	I	1.91 g	0.158g	1.59 f	1.38 e	0.275 d	40.7 g	25.3 g	23.7 g
	II	2.12 d	0.192d	1.80 c	1.49 c	0.330 c	48.0 d	30.3 d	31.3 d
SCU	0	2.05 e	0.180e	1.89 d	1.46 c	0.308 d	45.6 e	28.5 e	28.7 e
	I	2.23 a	0.222a	1.92 a	1.61 a	0.394 a	56.6 a	34.6 a	39.0 a
	II	2.24 a	0.225a	1.93 a	1.63 a	0.399 a	58.1 a	35.2 a	39.6 a
UF	0	1.98 f	0.170f	1.66 e	1.42 d	0.286 d	43.2 f	26.8 f	26.2 f
	I	2.18 c	0.201c	1.86 b	1.53 b	0.349 b	50.6 c	32.2 c	33.8 c
	II	2.31b	0.211b	1.91 a	1.57 b	0.365 b	53.1 b	33.8 b	36.2 b

**U: urea (46.5% N); UF: Urea formaldehyde (38.37% N); S.C.U.: Sulfur-coated urea. (40 % N)*
**Means in column followed by the same letters are not significantly different according to L.S.D. P = 0.05.*

Magnesium

It is evident from presented data that application of N in slow release forms was significantly preferable in increasing leaf Magnesium percentages than those treated with the fast release one. Moreover, treatments of SCU+ VAM at either level exerted similar trend that obtained for Calcium percentages (0.399 and 0.394 %) followed in descending order by UF with comparatively higher enhancing effect as combined with either VAM inoculated levels (0.349 and 0.365 %).

Micro-nutrient contents

Data presented in Table 3 show that different treatments resulted in improving leaf Fe**,** Zn and Mn status within the optimum range according to current guide lines, except those treated with U form either alone or as combined with low VAM level. The vice versa was significantly obtained in trees inoculated with VAM at high or low level and fertilized with SCU that exhibited highest values of leaf Fe, Zn and Mn contents. In this respect, UF fertilizer scored the second order as combined with VAM treatment at high level. The rest treatments produced lower positive effect for tested micro-nutrients.

The above mentioned results revealed that application of nitrogen fertilizers in slow release form and/or inoculated with VAM fungi actively transported macro and micro-nutrients up to the plants reflecting remarkable increment in mineral contents to be within or higher than the optimum guide lines range. In general, the higher followed by lower VAM inoculation levels showed best results as combined with SCU fertilizer (in particular), followed in descending order by UF fertilizer + high VAM level.

Results concerning effect of N fertilizer in slow release form are agree with those obtained by (He *et al.,* 2002; Eman *et al.,* 2009; Almagrabi and Abdelmoneim, 2012 and , Kandil *et al.,* 2010). In addition, the observed benefits of Sulpher application may be attributed to the increased availability of nutrients in trees fertilized with SCU (Boutros *et al.,* 1995;14, Morte *et al.,* 2001 and Abdou Soaud, 2011). The increased nutrient absorption by Mycorrhizal trees were in agreement with the findings of (Bâ *et al.,* 2001; Nelson and Achar, 2001; Gupta *et al.,* 2002 and Fernando Borie *et al.,* 2010). They reported that raising VAM inoculation level induced a remarkable and pronounced effect on leaf mineral content than those of sole fast release N fertilizers.

Fruit Physical characteristics:

Fruit weight :

Table (4) show that tested treatments enhanced fruit weight as compared with un- inoculated control. In this respect, SCU treatment produced statistically similar and highest positive effect on fruit weight of trees inoculated with high or low VAM level (178.6&178.9 *g*), as compared with the rest tested treatments. Additionally, trees inoculated with high VAM level and fertilized with UF arranged the third order in this respect.

Fruit numbers / Kg:

Data showed that fruit numbers were highly affected by slow release nitrogen fertilizer applications rather than Urea. The promotion effect was associated by increasing inoculation rate in Mycorrhizal trees. SCU treatment proved to be the efficient treatment in enhancing fruit number, as combined with either VAM level (5.60&7.5.59) with non significant differences. Moreover, raising VAM level inoculation enhanced UF treatment to reflect significant increase in fruit number (5.71) and arranged the third order, in this respect.

Fruit shape index (L/D)

It is obvious from Table (4) that tested nitrogen forms produced positive effect on either fruit length (**L**) or Fruit Diameter (D) as compared with the un-inoculated U control. According to the parallel increment in both fruit length (L) or Fruit Diameter (D), consequently Fruit shape index (L/D) ratios were attained in non significant differences for tested treatments.

Table 4: Effect of VAM inoculation and / or nitrogen forms on fruit physical characters of Valancia orange trees grown in calcareous soils. (Mean of two seasons)

N Form	VAM level	Fruit Wt. (g)	Fruit No. per each (*kg.*)	Fruit volume (*ml.*)	Peel thickness (*mm*)	Fruit dimensions(cm.)			Total Yield *Kg/tree*
						Fruit length (cm.)	Fruit diameter (cm.)	Fruit shape index (L/D)	
U	0	155.9gh	6.41 h	106 g	10.3 g	8.5f	7.84e	1.02e	84.1g
	I	158.9 g	6.29 g	110 f	10.8 g	8.9f	8.48d	1.05c	86.3f
	II	168.6d	5.931 d	121 d	12.5 d	9.9d	9.17b	1.08b	95.4d
SCU	0	177.2 e	5.64 e	116 e	12.0 de	9.4e	9.04b	1.04d	91.6e
	I	178.6 a	5.60 a	134 a	14.3a	11.2a	10.28a	1.09a	107.2a
	II	178.9 a	5.59 a	137 a	14.5 a	11.0a	10.28a	1.07b	109.6a
UF	0	162.2 f	6.17 f	113 ef	11.4 f	9.2e	8.93c	1.03d	88.5f
	I	171.9 c	5.82 c	126 bc	13.2 c	10.4c	9.46b	1.10a	101.4c
	II	175.1b	5.71 b	130 b	13.8 b	10.8ab	10.19a	1.06c	105.1b

U: urea (46.5% N); UF: Urea formaldehyde (38.37% N); S.C.U.: Sulphur - coated urea.(40 % N)
Means in column followed by the same letter are not significantly different according to L.S.D. test (P = 0.05).

Fruit volume

Tabulated data demonstrated that N forms of tested slow release fertilizers succeeded in enhancing fruit volume as compared with the traditional fast release nitrogen "control". The highest effect was significantly obtained as mycorrhizal trees at either level was treated with SCU (137&134 *ml*.). The efficiency of UF fertilizer exerted statistically higher enhancing effect as combined with high VAM level (130 *ml*.). Moreover, applying Urea to low Mycorrhizal trees failed to reflect any significant increase in this respect.

Peel thickness:

Regardless effect of Urea or U + low VAM level, data cleared a significant increase in peel thickness by different treatments. Maximum values were significantly obtained in trees inoculated with low or high VAM rate and fertilized with SCU (14.3&14.5 *mm*.) followed by UF fertilizer + high VAM rate (13.8 *mm*.). In this respect, UF + low VAM level surpassed the other tested treatments.

Total Yield /tree:

As shown in (Table 4), fertilization of valancia orange trees with SCU or UF fertilizers significantly increased fruit weight, fruit number/tree and consequently tree yield than those of quick release nitrogen fertilizer (control). The highest yield values were recorded with sole treatment of SCU (91.6 *Kg*) followed by UF treatments (88.5 *Kg*). In general, increasing inoculation level via either fast or slow release fertilizers resulted in increase yield components. While application of SCU fertilizers to low or high Mycorrhizal trees recorded the highest yield values (109.6 or 107.2 *Kg* per tree) followed in descending order by UF treatment as applied to mycorrhizal trees at high or low VAM levels (105.1*kg & 101.4 Kg*.), respectively. Though the high VAM rate was responsible for enhancing Urea effect to reach (95.4 Kg), the lower yield values were statistically obtained as U added to low Mycorrhizal trees (86.3 Kg). The remained treatments gave in between values in this respect.

Briefly, Mycorrhizal trees at both experimental levels proved to be the superior treatment in enhancing fruit physical characteristics as fertilized with SCU followed in descending order by UF treatment. The quick release nitrogen fertilizer "control" induced remarkable effect as added to trees with high VAM level.

fruit chemical contents:

Table, 5 show the effect of VAM inoculation and / or nitrogen sources on fruit edible rate%, SSC %, Total acidity, S.S.C/acid, Total sugars and Vitamin C contents of Valancia orange trees.

Total Soluble Solids Percentage (SSC%):

Data cleared that applying SCU or UF fertilizers, equally improved the fruit SSC contents compared to the fast release one. The highest SSC values were significantly found as high or low Mycorrhizal trees fertilized with SCU (13.2 or 13.1 %, respectively) followed in descending order by UF as combined with either high or low VAM level(13.0 or 12.9 %) with non significant differences. On the other hand, the remained treatments scored in between values in this concern.

Total acidity (%):

Results proved that all tested treatments succeeded in reducing fruit total acid content in comparison with the sole Urea treatment. Maximum values were obtained with sole application of U. Contrarily, high reduction effects were significantly recorded with those of SCU or UF as applyed to high mycorrhyzal trees (1.015 or 1.024). Also, Urea or UF + high VAM level reduced acid values to be nearly equal with those of SCU + low VAM level (1.025,1.024% 1.023%) , respectively.

S.S.C /Acid Ratio:

It is obvious from data that Mycorrhizal trees at low or high level produced fruits with highest S.S.C/ acid ratios as fertilized with N in SCU form (13.0 or 12.8), compared with other used treatments or sole U control . Moreover, UF + either VAM levels followed in descending order by Urea + high VAM level recorded higher significant values, in this respect.

Total Sugars (%):

Data cleared that both slow release fertilizers produced fruits rich in their total sugar contents than those produced by Urea fertilizer (control). The higher values were recorded in trees inoculated with VAM fungi at either level and fertilized by SCU to be the best in this concern (7.19 and 7.11 %). Also, UF exerted higher effect on fruit sugars as applied to trees with high VAM level (6.82 %) followed by UF + low VAM level(6.37 %).While, urea arranged the fourth as combined with high VAM level .

Table 5: Effect of VAM inoculation and / or nitrogen forms on fruit chemical contents of Valancia orange trees grown in calcareous soil. (Mean of two seasons)

N Form	VAM level	Edible rate %	SSC%	Total acidity TA%	Maturity index	Ascorbic acid mg/ 100 ml.	Total sugars g/100 ml juice (%)
					SSC/Acid		
U	0	72.2d	11.9fg	1.072a	11.1ef	28.8e	4.63 h
	I	72.5d	12.1ef	1.052b	11.5 e	31.4f	4.81 g
	II	73.6b	12.5cd	1.025e	12.2cd	33.9d	5.98 d
SCU	0	74.3ab	12.7c	1.033d	12.3 c	34.2c	5.68e
	I	76.9a	13.1a	1.023e	12.8 a	35.6a	7.11a
	II	76.4a	13.2a	1.015f	13.0 a	35.9a	7.19 a
UF	0	73.1c	12.3e	1.042c	11.8 d	32.6d	5.34 f
	I	74.5b	12.9b	1.032d	12.5bc	34.6bc	6.37 c
	II	74.9b	13.0ab	1.024e	12.7 b	35.1b	6.82 b

U: urea (46.5% N); U.F. :Urea formaldehyde (38.37% N) ; S.C.U.: Shlphur-coated urea. (40 % N)
Means in column followed by the same letter are not significantly different according to L.S.D. Test (P = 0.05).

Vitamin C content:

Tabulated data revealed that either SCU or UF fertilizers enhanced fruit citric acid content than control. Best results were similarly obtained by using SCU with both Mycorrhizal levels (35.9 & 35.6 *mg*). Also, V.C. content was significantly higher in fruits of highly mycorrhizal trees as fertilized by UF form (35.1 *mg*). Meanwhile, high VAM level proved to be essential for enhancing Urea fertilizer to produce pronounced values (33.9 *mg*) of V.C.

Fruit edible rate:

Table (5) shows that all tested treatments succeeded in increasing fruit edible rate in comparison with un-inoculated control. Applying SCU or UF to Mycorrhizal trees proved to be the best treatments. Where, SCU induced the highest significant effect as added to either Mycorrhizal level, followed in descending order by UF + high VAM level and UF + low VAM level. Meanwhile, Urea treatment increased fruit edible rate to high significant values as combined with high VAM level. The rest treatments scored in between values in this respect.

On the basis of obtained results, it may be concluded that fertilization of Valancia orange trees with high VAM rate improved tree growth, increased leaf mineral contents and fruit yield of high quality. This observation was markedly pronounced in trees fertilized with dual fertilizer (VAM and SCU). The prospective results for slow release N fertilizers on fruit properties, yield and fruit quality are in harmony with the findings of (Eman *et al.,* 2009; Ciprian and Iovu , 2010 and Morte *et al.,* 2001). Such observation may be due to the ability of VAM fungi to interact with other soil microbes like the free-living nitrogen fixers and phosphate solubilizers to improve their efficiency for the biochemical cycling of elements and supply the host trees with their nutrients requirements (Miller *et al.,* 1985 ; Boutros *et al.,* 1995; Bâ *et al.,* 2001; Tabassum *et al.,* 2012 and Almagrabi and Abdelmoneim, 2012).

Conclusively, application of the dual save bio-fertilizers (VAM fungi at high rate and N in Sulfur coated urea form) to Valancia orange trees is highly recommended to enhance tree growth and consequently produce high yield with good marketing quality.

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