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Comparing Physiological Role of L-Methionine and its Encapsulated Nano-Form on Growth and Crop Productivity of Onion (*Allium Cepa* L.)

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Abstract

Two field experiments were carried out at a private farm at Sakha Region, Kafr El-Sheikh Governorate, Egypt, during the winter seasons of 2020/2021 and 2021/2022. The experiment aimed to study the physiological response of onion plants to foliar spraying with methionine (50, 75 and 100 mg/L) and its nanoparticles (5, 10 and 15 mg/L). It is obvious from results that all applied treatments induced significant increases in most investigated parameters. The most significant and pronounced treatments were PEG- encapsulated methionine at 5mg/L followed by 100 mg/L methionine relative to control. It is worthy to mention that PEG- encapsulated methionine at 5mg/L significantly increased vegetative growth parameters (dry weight of leaves /plant by 144.62% and bulb fresh weight by 125.28 %); total soluble solids by 59.53%; total photosynthetic pigments by 210.81%; indole acetic acid by 54.34%; and yielded bulb fresh weight by 60.70 %. In addition, PEG- encapsulated methionine at 5 mg/L significantly increased total phenolic content by 1.18 times; flavonoid by 1.26 times; free amino acid by 1.92 times; protein by 1.37 times; and total soluble sugars by 1.66 times relative to control. The least increases were recorded due to methionine at 50 mg/L and PEG- encapsulated methionine at 15 mg/L relative to control. It could be concluded that PEG- encapsulated methionine at low doses (5 mg/L) may be used as promising technique to increase onion productivity and quality.

Keywords: Sulphur containing amino acid, *Allium cepa* L, fertilizer, nanoparticles

1. Introduction

The application of a lot of chemical fertilizer to ensure enormous crop yield leads to serious problems for the environment and products of agricultural. So, there is a necessary for applying appropriate horticultural approaches to replace chemical-based fertilizers. Recently, a great attention was focused on using organic fertilizers as amino acids. Amino acids are organically produced fertilizers that are easily absorbed, transferred, and used by plants due to their contents of nitrogen and carbon. Amino acids are better organic source of nitrogen that absorbed and assimilated by plants than its inorganic compounds [1]. Hence, plenty of researches have been conducted on the usage of amino acids to fertilize plants, particularly under harsh environmental circumstances [2]. Likewise, the administration of amino acids, as a sort of growth-promoting substance, ultimately boosts the crops yield and commercial output [3].

Generally, application of amino acids on plants improves nutrient availability, and enhances plant quality [4, 5]. Moreover, amino acids serve as hormone precursors, signaling factors of different physiological progress [6], and antioxidant metabolism [4].

L-Methionine is an essential sulfur-containing amino acid. Additionally to its contribution to protein synthesis, it plays an essential role in plant growth and development [7, 8]. The positive effects of application of L-methionine on plants can be clarified as follows (1) It plays a crucial role in preserving the structural integrity of proteins necessary for cell development, differentiation, and division. (2) It fulfills necessary sulfur and nitrogen in accordance

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with to plant demands [9]. (3) It serves as a buffer and behaves a reservoir of carbon, and source of energy [10] (4) It has the ability to be converted into polyamines [11]. (5) It acts as a precursor of many secondary metabolites [12]. (6) It promotes the plant growth by synthesis of cytokine in, brassinosteroids, and auxin [13, 14] which may promote the homeostasis of endogenous hormones [15]. (7) it serves as a precursor for an extensive array of vital biomolecules, including vitamins and antioxidants like glutathione, that are thought to be important regulators of cellular redox equilibrium [16].

Accordingly, Maqsood *et al*. [17] mentioned that exogenous application of L-methionine partially alleviated the water deficit induced inhibition of whole-plant growth and antioxidant metabolism by minimizing lipid peroxidation, reactive oxygen species and enhanced the biosynthesis of the endogenous antioxidant pool.

Nanoparticles (NPs) are small molecules with a tiny size range of 1–100 nm and they have different physiochemical properties from bulk molecules [18-20].The distinctive characteristics of NPs, such as their high surface area and powerful reactivity, make them easier to interact with plant tissue. Likewise the uptake and transport of NPs inside plants are greatly influenced by their size, concentration, stability, and chemical structure [20, 21]. Since, nano-fertilizers are more effective and efficient than traditional fertilizers due to very low application amounts, rapid uptake by plant cells, and their routes of transport and representation within plant tissue leading to positive effects on the quality of crops [22]. Though materials coated with nano particles are able to pass via a plant's stomata with a size restriction threshold that exceeds 10 nm, the NPs appear to be able to make holes and reach the vascular system. NPs are able to penetrate the plant system via the aerial pathway through hydathode, stomata, and trichome structures as well as through wounds [23]. Once the NPspass through the epidermis, the apoplastic or symplastic pathway is used to transport the NPs throughout the plant [24]. Various reports stated that NPs can be transported via the apoplastic pathway, where they penetrate plant tissue through cell wall pores and diffuse into the intracellular space between the cell membrane and cell wall [25]. Recent studies have suggested that exposure to NPs may trigger the pores of cell walls to expand and allow NPs to enter. On the other hand, certain authors have documented the translocation of NPs via the symplastic pathway, in which NPs penetrate the cell membrane and reach cytoplasm or neighboring cell wall pores referred to as plasmodesmata. The effect of NPs on plants is impacted by several factors, as the physical and chemical characteristics of NPs, plant species, applied concentration, and others [26, 27]. Nanomaterials have negative impacts on plants when utilized in higher amounts, whereas they have positive effects when used in lower levels [28]. Numerous NPs have demonstrated the ability to be utilized as activators of phytohormone biosynthesis, regulating plant growth and metabolism in responses to abiotic stress [29, 30] and enhancing plant growth and production [31]. It is worthy to mention that application of NPs foliar as foliar treatment can regulate growth, biomass production, and yield in several agricultural crops [32, 33]. Whereas, under another circumstances, it may cause deficiencies in nutrition, retard root elongation, and postpone flowering in others [34].

Onion (*Allium cepa* L.) is regarded as the third most valuable vegetable crop in Egypt after tomato and potato. Onion is a source of ascorbic acid and dietary fiber. It also has a high concentration of flavanoids (mainly quercetin and its conjugates) and sulphur compounds (i.e. thiosulphinates), both of which have a high level of antioxidant activity [35]. Onions are one of the plants that prefer sulphur fertilization to grow well, soundly and to give a good yield. This is because sulphur is involved in the formation of a number of sulfur compounds that give onions their distinct flavor, aroma, and spicy flavor [36]. As a vegetable, it significantly contributes to the human diet and has a therapeutic property. Therefore, increasing the productivity of onions with high quality is an important goal for onion growers.

This work focused on the physiological role of sulphur containing amino acid (methionine) and its nanoform on growth and productivity of onion crop.

2. Materials and Methods

Materials

We bought poly ethylene glycol PEG (MW 2000 kDa) from Sigma-Aldrich, USA. L-Methionine, (S)-2-Amino-4-(methyl mercapto) butyric acid, L-2-

Amino-4-(methylthio) butanoic acid, with Molecular Weight: 149.21, was acquired from Merck. Other substances (ethanol, acetic acid, and buffer sodium phosphate) were of analytical grade.

Preparation of PEG- encapsulated methionine

The encapsulation approach was used to create methionine that was enclosed. L-methionine-encapsulated PEG was created using five different ratios of L-methionine to PEG 2000 (1:0.5, 1:1, 1:1.5, 1:2, and 1:2.5). PEG was added to 10 milliliters of boiling water. L-Methionine was encapsulated by first combining methionine with an alcohol solution, then adding sodium phosphate using a high-speed homogenizer. For testing, the encapsulated methionine was freeze-dried.

Characterization of PEG- encapsulated methionine

FT-IR spectroscopy

The interaction of functional groups between methionine and PEG was examined using a Fourier transform infrared spectrophotometer (ATR-FTIR) by Bruker VERTEX 80 (Germany) joint Platinum Diamond ATR comprises a diamond disc as that of an internal reflector in the range 400-4000 cm-1 with resolution 4 cm-1, refractive index 2.4. For this purpose, KBr pellets were obtained and the lyophilized encapsulated methionine was pressed to a plate.

Measurement of particle size, zeta potential and Transmission electron microscope (TEM) imaging

The shape of PEG-encapsulated methionine was examined using TEM to characterize the encapsulated form of the amino acid. The lyophilized, encapsulated methionine was diluted to 1/100 (v/v) in deionized water and stained for one hour with 2% phosphotungstic acid (a negative stain) before being observed. Using a dynamic light scattering (DLS)-nanoparticle size analyzer and the zeta potential (surface charge) of the produced nanoparticles, the hydrodynamic mean diameter of the encapsulated methionine and their size distribution were calculated. TEM was used to further characterize the morphology of the PEG-encapsulated methionine nano-emulsion.

Determination of encapsulation efficiency

Calculating the encapsulation efficiency of PEG-encapsulated methionine that was loaded with methionine in ratios of 1:0.5, 1:1, 1:1.5, 1:2, and 1:2.5 allowed researchers to establish the optimal loading capacity for methionine during the encapsulation process. According to the formula EE% = Encapsulated methionine/Total methionine 100, the encapsulation efficiency (EE) of the PEG was calculated as the percentage ratio of the encapsulated to the total methionine. The difference between the total amount of methionine initially added and that of the free and encapsulated methionine was used to determine the amount of the encapsulated methionine. For this, nano-encapsulated methionine was separated from free methionine in the aqueous medium by ultracentrifugation at 40000 g for 45 min at 4 °C, and the free methionine in the supernatant was quantified using the nitroprusside technique 40. Briefly, 1 ml of the supernatant was combined with 0.5 ml of 1% sodium nitroprusside and 1 ml of 5N NaOH. After 10 minutes of incubation at room temperature, samples were exposed to photometric measurements at 520 nm in a UV-VIS spectrophotometer (Shimadzu UV 1601 spectrophotometer, Tokyo, Japan).

Experimental procedure

Two field experiments were carried out at a private farm at Sakha Region, Kafr El-Sheikh Governorate, Egypt, during the winter seasons of 2020/2021 and 2021/2022 to study the physiological response of onion plants to foliar spraying with methionine and its nano-form.

Onion seedlings (*cv*: Red onion) were obtained from Onion Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. On the 10^{th} and 12^{th} of December throughout both seasons, onion seedlings that were about 60 days old and around 25 cm in height were removed, tied, and transported to the permanent field. Along the ridge top, seedlings were arranged in rows (with shallow furrows). The recommended doses of chemical nutrients (P_2O_5, K_2O, Ca) were added once by broadcasting during soil preparation. Nitrogen fertilizer in ammonium nitrate form was added after transplanting in two doses at the first irrigation (25 DAT) and 30 days later. All the cultural procedures like nursery raising, main field preparation, transplanting, fertilization, irrigation; weeding, plant protection etc. were carried out as recommended.

During the two experimental seasons, foliar application with three concentrations of L-methionine

(0, 50, 75 and 100 mgL^{-1}) and nano-methionine (0, 5, 10, and 15 mgL^{-1}) were applied twice after 30 and 45 days from transplanting. Moreover, the experiment treatments were arranged in a complete randomized block design with six replicates.

Recorded data

Vegetative characters

Plant samples were taken randomly to measure the growth criteria such as plant height, number of leaves/plant, fresh and dry weight of leaves/ plant, neck diameter, bulb length, bulb diameter, fresh and dry weight of bulb/plant; total soluble solids at vegetative growth stage (75 day after transplanting).

Yield and Bulb characteristics

Yield and bulb criteria was determined at the harvesting time (150 days from transplanting) including bulb length, bulb diameter, bulb fresh and dry weight /plant; total yield of bulb (ton fed^{-1}). Dry matter % and moisture content percentage was determined according to the following equations

$$\textbf{Dry matter}\ \% = \frac{\textbf{Bulb DW}}{\textbf{Bulb FW}}\ \textbf{x 100}$$

$$\textbf{Moisture content}\ \% = \frac{(\textbf{Bulb FW} - \textbf{Bulb DW})}{\textbf{Bulb FW}}\ \textbf{x 100}$$

Whereas, FW is fresh weight and DW is dry weight.

Chemical constituents

Photosynthetic pigments and indole acetic acid contents were evaluated in fresh leaf tissues (75 days after transplanting) according to Moran [37] and Larsen *et al*. [38] respectively. Total soluble solids were determined in fresh juice using Refractometer (Shibuya-0-32, Japan). Onion bulbs were dried in oven at 70ºC and then finally ground to determine total phenolic compound [39]; flavonoids [40]; free amino acid [41]. Total protein of the yielded grains was determined according to Pedrol and Tamayo [42]. Total soluble sugars were extracted by the method of Homme *et al*. [43] and analyzed according to Yemm and Willis [44].

Molecular assay:

Soft, fresh and young leaves of *Allium* plants under study were used to isolate and extract the genetic material (DNA) using CTAB according to Khaled and Esh [45] modified method. A total of 10 RAPD primers were tried, but 5 RAPD primers with positive results Table (1) were used in this study. However, the primers were chosen upon a previously used with different plant groups (fenugreek, bean, lupine, chickpea, sorghum and soybean) and the primers with high polymorphism rate were selected.

In order to perform PCR based analysis, it was carried out within 15 µl reaction volumes containing 1 µl DNA of plant, 7.5 µL Master Mix, 1 µL template DNA and 1 µL primer.

The program of PCR was 94°C for 5 min for initial denaturation, 94°C for 1 min (40 cycles), 37ºC for 1 min, 72ºC for 2 min and final extension at 72ºC for 10 min. As soon as, the amplified products were electrophoresed on 1.5 % agarose gel in 1×TAE buffer. Then ethidium bromide was used to stain the gel that photographed using gel documentation system.

Table (1): Positive RAPD primers with their sequences that used in this study.

Primer Code	Sequence
OPB-5	TGCGCCCTTC
OPA-12	TCGGCGATAG
OPA-14	TCTGTGCTGG
OPP-7	GTCCATGCCA
OPN-4	GACCGACCCA

Amplified DNA amplicons using ISSR marker were classified as absent (-) or present (+) and total number of bands (TB), polymorphic bands (PB), monomorphic bands (MB), unique bands (UB) and percentage polymorphism (PB%) were calculated.

Statistical analysis

Analysis of variance was used to statistically examine the average data average of two seasons. The differences between means were calculated using (LSD) at 5% and Duncan test according to Silva and Azevedo [46].

3. Results

Characterization of encapsulated methionine

FTIR –Spectroscopy

The encapsulated methionine with PEG shell was evaluated using FTIR. Figure (1) displays the FTIR spectrum of L-methionine. Utilising the KBr pellet technique, which was performed at room temperature in the range of 4000 to 400 cm, the recording of FTIR of L-Methionine-PEG was ensured. There is a large band between 2913 and 3241 cm-1 in the high energy zone. Using the observed FTIR spectrum, it is confirmed that L-Methionine-PEG contains a variety of functional groups. The resulting symmetric N-H stretching is what causes the absorption peak to occur at 3241 cm-1. The development of the NH3+ symmetry stretching mode is what causes the absorption peak to reflect at 2913 cm-1. The O-H vibration gives rise to the band that has been measured at 2358 cm-1. The C = O stretching is what causes the peak to emerge at 1613 cm -1. The symmetric stretching vibrations of COO- are what cause the band to come out at a wavelength of 1318 cm-1. The amino acid NH3+ deformation may be seen at 1187 cm-1 in the spectrum. The peak noted at 949 cm -1 is corresponding to the C-C symmetric stretching. The NH3 torsion bands respectively give rise to the IR band at 544 cm-1. L-Methionine's zwitter ionic character is confirmed by the appearance of NH3+ and COO- groups in the FTIR spectra.

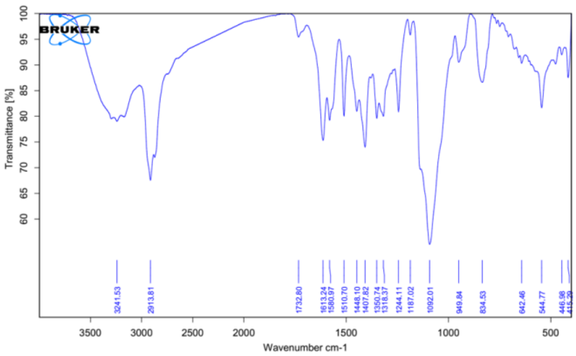

Figure (1): FTIR-of encapsulated methionine

Particle size, zeta potential, Transmission electron microscope (TEM) imaging and encapsulation efficiency

Using an encapsulation approach with PEG as the outer shell agent, a PEG-encapsulated methionine nano-emulsion was effectively created. Zeta Sizer Instrument used TEM and DLS to characterize a PEG-encapsulated methionine nano-emulsion. Data in table (2) demonstrated that decreasing particle size and improving encapsulation efficiency occurred with increasing the encapsulating aid PEG. The TEM picture (Figure 2) made it clear that the PEG-encapsulated methionine for recipe MP5 has a spherical form and an average size of 9–66 nm. According to the DLS results (Table 2), the PEG-encapsulated methionine nano-emulsion for recipe MP5 has a narrow size distribution with an average size of 70–80 nm. The swelling of PEG-encapsulated methionine nano-emulsion in aqueous solution may be to blame for the size discrepancy between prepared nanoparticles measured by TEM and DLS. DLS provides a hydrodynamic radius of nanoparticles, whereas TEM provides the dried diameter of prepared PEG-encapsulated methionine nano-emulsion. Additionally, DLS provides an overall image of the nanoparticles and their aggregations, whereas TEM provides an image for a chosen location for measurements. The PEG-encapsulated methionine nano-emulsion's zeta potential ranged from -20 to -40 mv. The anionic character of the preparation media caused the negatively charged PEG-encapsulated methionine nano-emulsion, which is a promising sign for the stability of the encapsulated emulsion. The data in Table (2) indicated that recipe MP5 had the best encapsulation efficiency, thus we chose to measure TEM for that recipe, which is shown in Figure (2).

Table (2): Particle size, zeta potential and encapsulation efficiency of PEG- encapsulated methionine nano-emulsion

Recipe	Me : PEG	P.S	Zeta potential	Encapsulation efficiency %
MP 1	1:0.5	500	-20	20
MP 2	1:1	380	-25	43
MP 3	1:1.5	170	-28	62
MP 4	1:2	175	-30	86
MP 5	1:2.5	70-80	-40	90

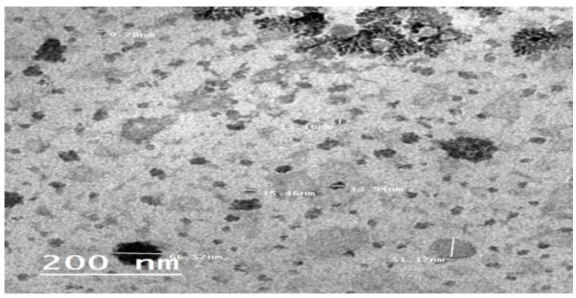

Figure (2): TEM for recipe MP 5 (average particle size 9-66nm)

Physiological parameters

Table (3A) shows the impact of methionine (50, 75 and 100 mg/L) and its nanoparticles (5, 10 and 15 mg/L) on vegetative growth parameters of onion plant (plant height; number of leaves/plant; fresh and dry weight of leaves/plant and neck diameter). It was noted that methionine at 75 and 100 mg/L and its nanoparticles at 5 and 10 mg/L induced significant increases in plant height and neck diameter relative to control. Whereas, all applied treatments significantly enhanced fresh and dry weight of leaves/plant relative to control. Effect of methionine increased as its concentration increased. Whereas, the effect of PEG- encapsulated methionine showed opposite trend. The most pronounced treatment was PEG-encapsulated methionine at 5mg/L followed by 100 mg/L methionine. Where, PEG- encapsulated methionine at 5mg/L significantly enhanced fresh and dry weight of leaves /plant by 154.68 and 144.62% respectively relative to control. Likewise 100 mg/L methionine significantly enhanced fresh and dry weight of leaves /plant by 149.30 and 119.52% respectively relative to control. PEG- encapsulated methionine at 15mg/L induced the least increases in all examined vegetative growth parameters relative to control

Table(3 A): Impact of methionine and its nanoparticles on vegetative growth parameters of onion plant

Treatments		Plant height (cm)	Number of leaves/plant	Leaves fresh weight/plant (g)	Leaves dry weight/plant (g)	Neck diameter (cm)
Control		61.67 d	7.00 c	25.31 d	2.51 e	1.43 b
Methionine (mg/L)	**50**	67.00 cd	8.67 abc	37.48 c	4.42 cd	1.53 b
	75	70.34 bc	8.67 abc	45.07 b	4.58 c	1.93 a
	100	77.00 b	9.00 ab	63.10 a	5.51 ab	2.10 a
Methionine Nps (mg/L)	**5**	86.67 a	10.00 a	64.46 a	6.14 a	2.10 a
	10	77.10 b	8.67 abc	50.77 b	5.07 bc	2.00 a
	15	61.67 d	7.67 bc	35.17 c	3.54 d	1.50 b
LSD at 5%		**7.77**	**1.79**	**5.93**	**0.89**	**0.33**

Table (3 B) shows that methionine (50, 75 and 100 mg/L) and its nanoparticles (5, 10 and 15 mg/L) induced marked increases in all examined vegetative growth parameters of onion plant (bulb length; bulb diameter; fresh and dry weight of bulb/plant, and total soluble solids). The least increases were recorded due to methionine at 50 mg/L and PEG-encapsulated methionine at 15 mg/L relative to control. Whereas, the highest significant increases were recorded due to PEG- encapsulated methionine at 5 mg/L followed by methionine at 100 mg/L. PEG-encapsulated methionine at 5 mg/L significantly increased bulb fresh weight by 125.28 %; bulb dry weight by 132.09 % and total soluble solids by 59.53% relative to control. On the other hand, methionine at 100 mg/L significantly increased bulb fresh weight by 102.75 %; bulb dry weight by 126.85% and total soluble solids by 45.98 %relative to control. It is obvious that the effect of PEG-encapsulated methionine at 5 mg/L was more prominent than methionine at 100 mg/L in inducing all examined vegetative growth parameters (Table 3 A and B).

Table (3 B): Ccontinued

Treatments		Bulb length (cm)	Bulb diameter(cm)	Bulb fresh weight/plant (g)	Bulb dry weight/plant(g)	Total soluble solid
Control		12.33 c	2.27 b	19.97 c	3.24 d	12.33 d
Methionine (mg/L)	**50**	13.00 bc	2.57 ab	30.19 b	4.47 c	16.00 bc
	75	14.33 ab	2.63 ab	30.65 b	4.78 c	17.00 b
	100	14.67 a	2.73 a	40.49 a	7.35 a	18.00 ab

Methionine Nps (mg/L)	**5**	15.00 a	2.77 ab	44.99 a	7.52 a	19.67 a
	10	14.67 a	2.63 ab	31.88 b	6.55 b	18.00 ab
	15	12.67 c	2.40 ab	29.93 b	3.39 d	16.00 bc
LSD at 5%		**1.42**	**0.49**	**5.58**	**0.47**	**2.09**

The physiological response of total photosynthetic pigments and IAA to methionine (50, 75 and 100 mg/L) and its nanoparticles (5, 10 and 15 mg/L) is shown in Table 4. All applied treatments significantly increased total photosynthetic pigments and IAA of fresh leaf tissues of onion plant relative to control. The most pronounced treatment was PEG-encapsulated methionine at 5 mg/L followed by methionine at 100 mg/L. Since, PEG- encapsulated methionine at 5 mg/L significantly increased total photosynthetic pigments by 210.81% and IAA by 54.34%. Whereas, methionine at 100 mg/L significantly increased total photosynthetic pigments by 196.52 % and IAA by 50 %. On the other hand the least significant increases in total photosynthetic pigments were recorded due to methionine at 50 mg/L. meanwhile; the least significant increases in IAA were recorded due to PEG- encapsulated methionine at 15 mg/L.

Table (4): Impact of methionine and nanoparticles on photosynthetic pigments (mg/g fresh leaf tissues) and indole acetic acid (IAA, mg/100 g fresh leaf tissues)

Treatments		**Chlorophyll a**	**Chlorophyll b**	**Carotenoid**	**Total photosynthetic pigments**	**IAA**
Control	**0**	0.141 e	0.061 e	0.057 e	0.259 e	0.92 e
Methionine (mg/L)	**50**	0.161 d	0.066 d	0.064 e	0.291 e	1.27 cd
	75	0.228 f	0.139 f	0.036 f	0.403 c	1.28 c
	100	0.461 b	0.150 a	0.157 b	0.768 b	1.38 b
Methionine Nps (mg/L)	**5**	0.483 a	0.148 a	0.174 a	0.805 a	1.42 a
	10	0.332 c	0.110 b	0.012 c	0.454 d	1.36 b
	15	0.176 d	0.083 c	0.077 d	0.336 f	1.24 d
LSD at 5%		**0.019**	**0.005**	**0.008**	**0.023**	**0.04**

The impact of methionine and its nanoparticles on onion yield and its attributes is shown in Table 5. All applied treatments caused marked increases in bulb length, bulb diameter, fresh and dry weight of bulb / plant; and dry matter accompanied by decreases in moisture content. The highest significant increases were recorded due to PEG- encapsulated methionine at 5 mg/L followed by methionine at 100 mg/L. PEG-encapsulated methionine at 5 mg/L significantly increased bulb fresh weight by 60.70 %; and bulb dry weight by 102.11 % relative to control. On the other hand, methionine at 100 mg/L significantly increased bulb fresh weight by 55.45 %; and bulb dry weight by 97.78% relative to control. It is obvious that the effect of PEG- encapsulated methionine at 5 mg/L was more prominent than methionine at 100 mg/L in inducing all onion yield and its attributes.

Table (5): Impact of methionine and its nanoparticles on onion yield and its attributes

Treatments		**Bulb length(cm)**	**Bulb diameter (cm)**	**Bulb fresh weight/plant (g)**	**Bulb dry weight/plant (g)**	**Dry matter (%)**	**Moisture content (%)**
Control		6.58 b	5.31 c	95.12 f	9.48 d	9.96 bc	90.03 ab
Methionine (mg/L)	**50**	7.10 b	6.80 b	128.36 d	12.50 c	9.74 c	90.26 ab
	75	7.11 b	6.91 b	134.91 c	13.63 bc	10.10 abc	89.89 abc
	100	7.38 ab	7.37 a	147.87 b	18.75 a	12.68 a	87.32 bc
Methionine Nps	**5**	8.17 a	7.67 a	152.86 a	19.16 a	12.53 a	87.46 bc

(mg/L)	**10**	7.24 b	7.35 a	135.70 c	16.44 ab	12.11 a	87.88 bc
	15	6.60 b	6.69 b	106.65 e	12.47 c	11.69 b	88.30 c
LSD at 5%		**0.87**	**0.40**	**6.51**	**2.95**	**1.67**	**1.67**

The changes in some biochemical composition of onion bulb(total phenolic content; flavonoid; free amino acids, protein content, and total soluble sugars) to methionine (50, 75 and 100 mg/L) and its nanoparticles (5, 10 and 15 mg/L) is shown in Table 6. All applied treatments induced marked increases in biochemical constituents of onion bulb. The highest significant increases in these biochemical constituents were recorded due to PEG- encapsulated methionine at 5 mg/L followed by methionine at 100 mg/L. PEG- encapsulated methionine at 5 mg/L increased total phenolic content by 1.18 times; flavonoid by 1.26 times; free amino acid by 1.92 times; protein by 1.37 times; and total soluble sugars by 1.66 times relative to control. The least increases were recorded due to methionine at 50 mg/L and PEG- encapsulated methionine at 15 mg/L relative to control.

Table (6): Impact of methionine and its nanoparticles on some biochemical composition of onion bulb

Treatments		**Total phenolic content(mg /g DW)**	**Flavonoid (mg /g DW)**	**Free amino acid (mg /g DW)**	**Protein content (%)**	**Total soluble sugar (mg /g DW)**
Control		5.19 b	4.64 d	20.99 e	13.50 e	33.99 c
Methionine (mg/L)	**50**	5.40 b	5.23 c	31.90 c	16.25 cd	52.53 b
	75	5.45 b	5.46 bc	33.50 c	16.50 bcd	53.99 ab
	100	5.57 b	5.70 ab	39.01 a	17.50 ab	54.94 ab
Methionine Nps (mg/L)	**5**	6.12 a	5.88 a	40.30 a	18.50 a	56.61 a
	10	5.49 b	5.76 ab	36.30 b	17.25 bc	54.66 ab
	15	5.34 b	4.86 d	28.49 d	15.50 d	52.76 ab
LSD at 5%		**0.42**	**0.32**	**2.46**	**1.07**	**3.99**

RAPD- Molecular Markers

Tables (7 and 8) show the effect of both methionine and its nano-particles on reproducible DNA fragments of onion plants. In this study 10 RAPD primers were used for polymorphism screening, out of which only 5 primers were found polymorphic (Table 1). However, multiple fragments with different molecular weights were detected using these primers, and the reproducible fragments distributed between polymorphic, monomorphic and unique bands.

It was noticed that the total detected bands **(TB)** were 82 bands that distributed as 43 polymorphic **(PB)** bands and had (87.81%) polymorphism. The highest level of polymorphism could be observed with primers **OPP-7** primer that showed (90.48%) polymorphism, while the lowest polymorphism was 84.62% with both primers **OPB-5** and **OPN-4** (Table 7).

Table (7): Impact of methionine and its nanoparticleson RAPD- markers of onion plants

Primers	**Marker size (bp)**	**Amplified bands**				**PB %**
		TB	**PB**	**MB**	**UB**	
OPB-5	76.32 – 416.67	13	6	2	5	84.62
OPA-12	104.50–507.97	19	9	2	8	89. 47
OPA-14	116.86 – 464.36	16	9	2	5	87.50
OPP-7	164.47 - 580.18	21	10	2	9	90.48
OPN-4	129.37 – 507.49	13	9	2	2	84.62
Total		82	43	10	29	87.81
Average		16.4	8.6	2.0	5.8	-

Moreover, the detected bands were varied in number, polymorphism and range of its molecular weights between used RAPD primers. With regard to primer **OPB-5,** 13 bands were detected with this primer and molecular weights of these bands ranged between (**76.32 – 416.67bp)**.Moreover, it distributed as 2 monomorphic bands **(MB)**, 5 unique bands **(UB)** and 6 polymorphic bands **(PB)** with 84.62% polymorphism. Meanwhile, there were 19 bands with molecular weights (**104.50– 507.97bp**) and 89.47% polymorphism were detected using**OPA-12** primer, and distributed as 2 **(MB)**, 8 **(UB)** and 9 **(PB).** Moreover, there were 16 bands with molecular weights (**116.86 – 464.36bp**) and 87.50% polymorphism were detected using**OPA-14** primer, and distributed as 2 **(MB)**, 5 **(UB)** and 9 **(PB) (**Table 6).

On the other hand, the highest total amplified bands (21), polymorphic bands (10), unique (9) and polymorphism% (90.48%), respectively, were scored with**OPP-7 primer** with molecular weights ranged between (**164.47 - 580.18**)**(**Table 7 and Figure 3).

Table (7) represent a general idea about the reproducible bands detected using previous five RAPD primers. However, Table 8 draws the attention to number, size, type and conjugative reproducible bands that were detected by each primer separately. Moreover, there were some bands which have the same molecular weight and these called polymorphic bands and this conjunction due to the effect of the treatments.

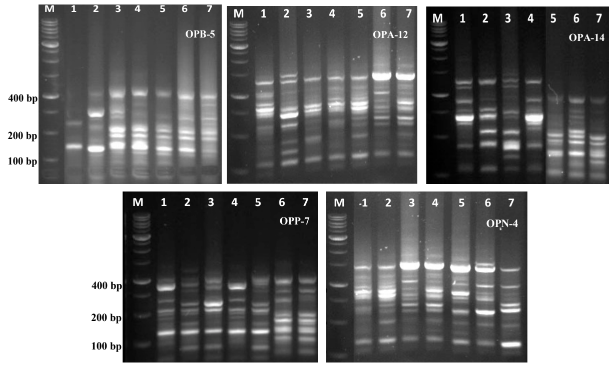

Fig. 3: Impact of methionine and its nanoparticleson RAPD- markers of onion plants

M=DNA Marker; 1=control; 2=Methionine (50mgL^{-1}); 3=Methionine (75mgL^{-1}); 4= Methionine (100mgL^{-1}); 5= Methionine NPs (5mgL^{-1}); 6= Methionine NPs (10mgL^{-1}); 7= Methionine NPs (15mgL^{-1}).

Table (8): Impact of methionine and its nanoparticles foliar spraying on RAPD- DNA of onion plants

MW	Cont.	Meth. (50 mgL^{-1})	Meth. (75 mgL^{-1})	Meth. (100 mgL^{-1})	Meth. NPs (5mgL^{-1})	Meth. NPs (10 mgL^{-1})	Meth. NPs (15 mgL^{-1})	Polymorphism
OPB-5								
416.67	+	+	-	-	-	-	-	PB
337.68	+	+	+	+	+	+	+	MB
298.89	-	-	+	-	-	-	-	UB
291.55	-	-	-	+	-	-	-	UB
287.62	-	+	-	-	-	-	-	UB
260.40	-	-	-	-	+	+	+	PB
224.82	+	-	-	-	-	-	-	UB
195.42	-	+	+	+	+	+	+	PB
169.87	-	-	+	+	+	+	+	PB
151.38	+	-	-	-	-	-	-	UB
134.59	-	+	+	+	+	+	+	PB
98.31	-	+	+	+	-	-	-	PB
76.32	+	+	+	+	+	+	+	MB
OPA-12								
507.97	-	+	+	+	+	+	+	PB
460.23	+	+	-	-	-	-	-	PB
443.51	-	-	-	-	-	-	+	UB
433.59	-	+	+	+	-	-	-	PB
401.00	-	-	+	-	-	+	-	PB
353.94	+	-	-	-	-	-	-	UB
324.47	+	+	+	+	+	+	+	MB
272.44	-	+	-	-	-	-	-	UB
265.80	-	-	-	-	+	+	+	PB
239.83	-	+	+	+	+	+	+	PB
226.88	+	-	-	-	-	-	-	UB
211.12	-	-	-	+	-	-	-	UB
204.71	-	-	-	-	-	-	+	UB
196.46	-	-	-	-	+	-	-	UB
175.82	+	-	-	-	-	-	-	UB
165.30	-	+	+	+	-	-	-	PB
147.32	+	+	+	+	+	+	+	MB
126.79	-	+	+	+	-	-	-	PB
104.50	+	-	-	-	-	-	+	PB
OPA-14								
464.36	+	+	+	+	-	-	-	PB
410.92	+	-	+	-	-	-	-	PB
334.47	+	-	-	-	-	-	-	UB
333.79	-	-	-	+	+	+	+	PB
307.66	+	-	-	-	-	-	-	UB
291.78	-	+	+	-	-	+	-	PB
277.86	-	-	-	-	-	+	-	UB
262.44	+	+	+	+	-	-	-	PB
237.02	-	-	-	-	+	+	+	PB

211.89	-	+	+	-	+	+	-	**PB**
196.50	+	-	-	-	-	-	-	**UB**
187.50	-	-	-	-	-	-	+	**UB**
173.88	+	+	+	+	+	+	+	**MB**
160.62	-	-	-	-	+	+	+	**PB**
146.52	-	-	-	-	+	+	+	**PB**
116.86	+	+	+	+	+	+	+	**MB**
OPP-7								
580.18	-	+	-	-	-	-	-	**UB**
512.39	+	-	-	-	-	-	-	**UB**
504.81	-	-	+	-	-	-	-	**UB**
484.33	-	-	-	-	+	+	+	**PB**
459.33	+	+	+	+	-	-	-	**PB**
423.52	-	+	-	-	-	-	-	**UB**
414.50	-	-	-	-	+	+	+	**PB**
384.72	+	-	-	-	-	-	-	**UB**
364.26	-	+	+	+	-	-	-	**PB**
347.17	-	-	-	-	+	-	-	**UB**
325.45	+	+	+	+	+	+	+	**MB**
311.22	-	-	-	-	+	+	+	**PB**
298.10	-	-	-	+	-	-	-	**UB**
283.18	+	-	-	-	-	-	-	**UB**
270.79	-	+	-	-	+	+	+	**PB**
248.03	+	+	+	+	+	+	+	**MB**
220.51	-	-	-	-	-	+	+	**PB**
208.09	+	-	-	-	-	-	-	**UB**
195.40	-	+	+	-	-	-	-	**PB**
189.97	-	-	-	-	+	+	+	**PB**
164.47	+	+	+	+	+	-	-	**PB**
OPN-4								
507.49	+	+	+	+	+	+	+	**MB**
435.39	+	+	-	+	+	-	-	**PB**
358.58	+	+	+	-	-	-	-	**PB**
347.05	-	-	-	-	+	+	+	**PB**
316.56	-	-	-	-	+	-	-	**UB**
301.42	+	+	+	-	-	-	-	**PB**
282.92	-	-	-	+	+	+	-	**PB**
252.33	-	-	-	-	-	-	+	**UB**
246.73	+	+	+	+	-	-	-	**PB**
228.76	+	+	+	+	-	-	-	**PB**
223.68	-	-	-	-	+	+	+	**PB**
186.49	-	+	-	+	+	+	+	**PB**
129.37	+	+	+	+	+	+	+	**MB**

4. Discussion

First of all, it is worthy to mention that amino acids are actually participated in all metabolic, regulatory, and physiological aspects of plant metabolism, and act as a source of carbon, nitrogen, energy, enzyme, and co-enzymes [12]. In addition, amino acids are well known bio-stimulant that act as precursors of several plant growth regulators and improve plant growth via increasing photosynthesis [47], production of sugar, protein and transcription of mRNA [48], and activate plant metabolic processes. Earlier studies showed that amino acids application promoted uptake of nutrients, enhanced the photosynthesis of hot pepper [49], flowering, fruit set, and fruit yield of tomato crops [50]. Another possible mechanism of amino acid could be related to promotion the root growth which improve uptake of water and nutrient, leading to boost cell division and increase fresh and dry matter and improve productivity of the yield [15]. Moreover, the improvement of plant growth and crop yield due to moving photosynthates to the photosynthesis machinery and increased rate of photosynthesis and leaf growth [56].

Regarding nitrogen effect, nitrogen is one of the major plant nutrients that plays crucial function in enhancing the yield and quality of fruits as well as the growth and development of plants. It is also necessary for the synthesis of enzymes and is a constituent of proteins and nucleic acids [57]. In addition, nitrogen plays an important role in photosynthesis and growth because of its necessary role in cell division and expansion, besides nitrogen is a constituent of chlorophyll, thylakoid proteins and enzymes of the photosynthetic process [58].

Regarding sulphur effect, Korsheed and Altememe [59] mentioned that application of sulphur on onion plants had a significant impact on plant growth and enhanced quantitative and qualitative of onion yield. Since, the enhancement in vegetative growth of onion that occurred as a result of spraying with organic sulphur may be attributed to the increased up take of sulphur by the onion plants, which play essential role in increasing cell divisions in the meristematic areas [36]. Moreover, Mahmoud and El-Tanahy [60] added that sulphur application increased yield of onion bulb and improved its quality, particularly pungency and flavors.

Earlier reports indicated that L-methionine not only make nutrients available to plants but also act as signal transducing molecules [61], where small doses of L-methionine are sufficient for plant development, and can act as signals of several beneficial plant physiological processes [3]. Ahmad *et al*. [58] stated that the application of L- methionine greatly increased relative growth rate, total leaf area, net photosynthesis and leaf nitrogen concentration of amino acids application, may be attributed to their role in increases chlorophyll biosynthesis and photosynthesis rate [2, 51, 52]. It was noted that application of amino acid individually acts as a signaling component, i.e., increased antioxidant enzyme activity and nutrient uptake [53].

L-methionine amino acid acts as source of carbon, nitrogen and sulphur and documented as source of plant growth regulator ethylene, which plays fundamental roles in various aspects of growth and photosynthesis [54].

Regarding ethylene effect, it can be suggested that ethylene acts as growth stimulator at low concentrations [55], stimulated photosynthesis by wheat. They added that the increase in photosynthesis by the application of L- methionine at 30 mg kg^{-1} soil may be attributed to the increase in stomatal conductance and higher photosynthates accumulation. Khan *et al*. [3] showed that L-methionine can be used as a suitable substitute for fertilizers to increase lettuce plant growth and crop yield, since it significantly improved growth performance by 23.60% via applying L-methionine at the lowest concentrations (0.2 mg/L). They added that L-methionine increased the chlorophyll content of plants and contributes to energy conservation, thus enhancing the plant yield. Moreover, application of L-methionine influenced phytohormones (e.g., auxins and cytokinin), which increased the chloroplast development and chlorophyll content [62]. Recently, Mehak *et al*. [63] stated that exogenously applied L-methionine was effective in minimizing the malondialdeyhde and hydrogen peroxide contents, and increasing the plant growth by increasing the shoot length, shoot fresh weight, chlorophyll pigments, proline contents and activity of antioxidant enzymes in sunflower plant grown under drought. They proposed using L-methionine as one of the efficient plant growth regulators under stressful environments.

Regarding chemical composition of the yielded crop, the application of amino acid on plant play an important role in the synthesis of plant compounds such as enzymes, proteins, amines, secondary metabolites, phenolic compounds and flavonoids, which regulate different plant processes [64]. In addition, the plants supplied with amino acids generally have higher amount of proteins, sugars, and other nutrients [2]. It is well-known that amino acids are the precursors of phenolic compounds [47].

Mobini *et al*. [65] demonstrated that amino acids spraying have positive effects on protein, phenolic and flavonoid compounds. Recently, administration of amino acid at 300 mg/L can be advised to enhance cabbage growth, productivity, biochemical features, and nutritional value processes [64].

Regarding role of Nps, it is well known that crops can slowly absorb nutrients from traditional fertilizers; meanwhile nano-fertilizers provides high surface areas to volume ratio leading to a greater number of active sites for biological activity [66]. Moreover, nano-fertilizers were more efficient and effective than the conventional fertilizers due to their positive effects on the growth, development and nutritional quality of different crops [22]. Recently, Swathi *et al*. [67] mentioned that leaf stomatal apertures facilitate the uptake of Nps and their entrance into leaves as a result of foliar application. According to Mala *et al*. [68], nano-fertilizer application promoted germination and biochemical features of *Vigna radiata* in comparison with traditional fertilizer since nano-fertilizers provided the crop plant with more nutrient availability that accelerating the rate of reaction or synthesis process and thereby enhanced the nutritional parameters of the plant. Hemerly [69] reported that the nitrogen application in nano form increased the plant growth, and yield quality by enhancing protein contents, and absorption of essential nutrients. Moreover, application of NPs at appropriate concentration have beneficial effects on plants including increases of yield quality, and decrease of oxidative stress [27] but sometimes high concentrations have adverse effects on crops [71]. NPs also showed significant increments on carotenoids, phenolics compound, indoletotal sugar, and amino acid contents of coriander and maize [72, 73]. Phenolic compounds are known as antioxidant compounds, which exhibit free radical scavenging activity due to the free hydroxyl group in their structure [74]. Therefore, the increased accumulation of total phenolic and flavonoids, indicated a positive effect of this L-methionine and its nano-particle on antioxidant capacity of onion plants.

Considering the importance of RAPD and ISSR markers for the study of genetic diversity in various genera and species of plants, very little work has been done in different species varieties and cultivars of onion [75]. So, this work is an attempt to study the effect of foliar spraying with methionine and methionine Nps on onion plants via changes in the reproducible RAPD-DNA fragments.

Hence, the changes in the reproducible RAPD-DNA fragments that detected as a result to the effect of foliar spraying with methionine and methionine nano-particles on onion plants were shown in Tables (7 and 8) and illustrated in Figure (3). Sudhaa *et al.* [76] and Mansour *et al.* [77] studied the genetic similarity and diversity between onion cultivars using molecular markers of RAPD. They found 52 and 32 total amplified polymorphic fragments respectively that produced via using RAPD primers.

You will usually want to divide your article into (numbered) sections and subsections (perhaps even subsubsections). Code section headings using the options in the 'Text' menu. Headings should reflect the relative importance of the sections. Note that text runs on after a 4th order heading. Use the heading style for the whole paragraph, but remove the bold coding except for the actual heading.

5. Conclusions

Modernistic, nano-technology had proved its position in agriculture. Moreover, no researches deal with application of nanoparticles of any amino acid on growth and productivity of different crops. So, this work aimed to investigate one of sulphur containing amino acid called methionine in its bulk form or nanoform on growth and productivity of onion plants. It could be concluded that foliar spraying with methionine (50, 75 and 100 mg/L) and its nanoparticles (5, 10 and 15 mg/L) induced growth and yield of onion by variable degree. The most significant and pronounced treatments were methionine nanoparticles at 5mg/L followed by 100 mg/L methionine relative to control. The least increases were recorded due to methionine at 50 mg/L and methionine nanoparticles at 15 mg/L relative to control. Methionine nanoparticles at low doses (5 mg/L) may be used as promising technique to increase onion productivity and quality.

Future work

1. To change the ratios of methionine in the recipe and investigate the stimulatory impact on plants cultivated under normal and salinity stress conditions.
2. Using different polymers as shell and incorporate other stimulator such as chitosan and curcumin

3. 3-Using natural polymer to improve sustained release of Methionine.

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Declarations
Ethics approval and consent to participate
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Consent for publication
The participants declare that the work has been consented for publication.

Competing interests
The participants declare that they have no competing interests.

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