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Research Article

Silver Nanoparticles as Catalysts of *Foeniculum vulgare* L. Callus Formation and Its Content of Vitamins and some Fatty Acids

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Background: Plants are a precious resource of a wide range of secondary metabolites, that are benefitted as flavours, pharmaceuticals, colours, colognes, food additives and also biopesticides.

Objective: The current study tested the impact of silver nanoparticles (AgNPs) on *Foeniculum vulgare*.

Materials and Methods: *Foeniculum vulgare* seeds were surface sterilized, Vital leaf pieces are grown on MS media including diverse mixtures of plant growth regulators, the special effects of AgNPs plus PGRs on callus propagation were evaluated, and separated compounds of fatty acid and vitamins were identified.

Results: Outcomes revealed that several intensities of AgNPs expressively influenced the callus propagation and significantly raised the callus biomass with combination including the plant growth regulators. Highest fresh (7.32 $g.L^{-1}$) biomass addition of callus was remarked on the media elevated *in vitro* at 20 ppm AgNPs combined with (2 $mg.L^{-1}$ 2,4-D) and results noted that the callus appeared compact and greenish in colour with 40 ppm AgNPs in combination with (2 $mg.L^{-1}$ 2,4-D). The results elucidated the amplification of the value of both fatty acids (stearic acid (47.85 %), oleic acid (189.28 %), Linoleic (6.34 %) and Linolenic (0.83 %)), and vitamins (Vitamin E (8.99 $U.mg^{-1}$) and vitamin A (27.19 $U.mg^{-1}$) by using MS + 2,4-D (2 $mg.L^{-1}$) + AgNPs (20ppm).

Conclusion: Application of a combination of AgNPs along with PGRs led to callus proliferation in *Foeniculum vulgare* L. *In vitro*. But, the unaccompanied use of AgNPs was originate inductive in the biosynthesis of greater quantities of special fatty acids and vitamin metabolites.

Keywords: Callus culture, Fatty acids, *Foeniculum vulgare*, Silver nanoparticles,Vitamins

1. Background

Silver nanoparticles (AgNPs) are considered as the most significant nanoparticle in exhaust product supplies due to their common antimicrobial charactristics alongside their effectiveness in private carefulness creations, food advantage, and medical tools (1). In recent years, Nanotechnology expanded quickly impacting various fields, namely: natural environment and economy. Silver nanoparticles are definitely the best expended nano composites amongst commercially accessible nano-sized substances, owing to their effective antimicrobial activity (2). Several researches confirmed that AgNPs

are extremely deadly to bacterial cell, algae, and fish, in addition to other living organisms, In spite of this, it stays challenging to totally realize the process of AgNPs harmfulness in continental plants life due partial findings. Recognizing plant reaction to AgNPs stress is valuable. In addition, it is essential for creating stress tolerant yields. Several research papers have exposed silver nanoparticles biosynthesis via plant extract with pharmacological capacity (3, 4). On the other hand, plant cell cultures application as a biotechnological implement is a viable substitution allowing the creation of enough aggregates of plant mass for the biological construction of nanoparticles, which is characterized as harmless and pure technique (5). For example, the biomass of certain varieties for instance *Medicago sativa* (6), *Jatropha curc* (7), and *Cucurbita máxima* (8), has been handled to manu-facture AgNPs by callus culture sources. Vitamins may work like enzyme cofactors (e.g., most B vitamins and vitamin K), natural antioxidants (e.g., vitamins E and C), hormones (e.g., vitamins D and A) (9).

Bionanotechnology has appeared as an integration between nanotechnology and biotechnology for the purpose of developing biosynthetic and environmental-friendly technology for the synthesis of nanomaterials. Nanochemistry at present time has become one of the major growing directions of nanosciences (10,11). Fennel (*Foeniculum vulgare* L.) is biennial plant of the Apiaceae (Umbelliferae) family which is broadly utilized to impart flavour to a number of foods; like pickles, soups, sauces, breads, cakes and also. It is usually used like digestive,carminative, lactogogue and diuretic and in curing respirational and gastric syndromes. Many compounds like phenolic glycosides, Phenols and volatile aroma compounds for instancetrans-anethole, estragole and fenchone had been described in the role of the chief phytocomponents of this types. Thus, diverse pharmacuticall investigates in a enumerous of *in vitro* and *in vivo* kinds have influentially confirmed the capability of *F. vulgare* for exhibition of antifungal compounds, antibacterial compounds, antioxidant compounds, antithrombotic compounds and hepatoprotective actions. (12). Also, survey of the special effects on plant tissue culture by NPs is rare. Particular papers in this area have in-dicated that plant callus may synthesise nanoparticles; (5,6). Other researchers have exercised NPs *in vitro* biological system decontamination for plant proliferation (6). The current paper has reasonably examined the influence of special Silver nanoparticles (AgNPs) concentrations in addition to plant growth regulators (PGRs) on Fennel (*Foeniculum vulgare* L.) tissue cultures, seeking to find the potential character of AgNPs, in stimulating or decreasing callus pro-pagation and/or shoot development and values of fatty acids and vitamins.

2. Objective

The objective of the current study was testing the impact of silver nanoparticles (AgNPs) on *Foeniculum vulgare* L plant and biosynthesis of greater quantities of special fatty acids and vitamin metabolites.

3. Materials and Methods

3.1. Seedlings Growth Condition

Foeniculum vulgare seeds were surface sterilized by immersing them for 2 minutes in ethyl alcohol (96%), then immersing them in NaOCl (5%) for five minutes, and then washing them by sterile distilled water (13). Sterile seeds were transferred into beaker including 25 mL of agar solid MS medium free of plant growth regulators (PGRs). Samples were kept in tissue culture room at 23 °C under 2000 lux light at (16/8) light/hour.

3.2. Callus Induction

Leaf fragments 0.5–1 cm are grown in flasks including MS media consisting of 3% sucrose, 0.8% and agar at pH 5.8 (14) using various mixes of PGRs (**Table 1**), at 24 ± 2 °C with a photoperiod of 16/ 8 hours (15).

Table 1. Callus induction from leaves of *Foeniculum vulgare* L. on MS medium complemented by mix of both 2,4-D and Benzyl adenine.

Hormonal combination	Abbreviation
MS0	C (Control)
MS +1 mg.L^{-1} 2,4-D	1D
MS +2 mg.L^{-1} 2,4-D	2D
MS +1 mg.L^{-1} BA	1B
MS +2 mg.L^{-1} BA	2B
MS +1 mg.L^{-1} 2,4-D + 1 mg.L^{-1} BA	D, B
MS +2 mg.L^{-1} 2,4-D + 2 mg.L^{-1} BA	D, B

Table 2. Impact of AgNPs unaccompanied or with 2,4-D on proliferation of callus in *Foeniculum vulgare* L.

Treatment		Fresh weight /g
MS0	Mean	0.8490 f
	N	3
	Std. Deviation	0.00173
MS+2,4-D(2mg.L^{-1})	Mean	1.0650 e
	N	3
	Std. Deviation	0.00500
MS+AgNPs(20ppm)	Mean	3.1160 c
	N	3
	Std. Deviation	0.01039
MS+AgNPs(40ppm)	Mean	2.8447 d
	N	3
	Std. Deviation	0.04225
MS+2,4-D(2 mg.L^{-1})+AgNPs(20ppm)	Mean	7.3200 a
	N	3
	Std. Deviation	0.03464
MS+2,4-D(2 mg.L^{-1})+AgNPs(40ppm)	Mean	4.5800 b
	N	3
	Std. Deviation	0.08000
Total	Mean	3.2958
	N	18
	Std. Deviation	2.26413

Table 3. Impact of AgNPs unaccompanied or with 2,4-D on callus morphology in *Foeniculum vulgare* L.

Treatment	Callus nature	Callus colour
MS0	Fragile	Greenish
MS+2,4-D(2mg.L^{-1})	Fragile	Yellow greenish
MS+AgNPs(20ppm)	Fragile	Yellow greenish
MS+AgNPs(40ppm)	Compact	Greenish
MS+2,4-D(2mg.L^{-1})+AgNPs(20ppm)	Compact	Yellow brownish
MS+2,4-D(2mg.L^{-1})+AgNPs(40ppm)	Semi-Compact	Greenish

3.3. Purpose of Adding AgNPs Upon Callus Biomass Growth and Proliferation

To assess the influence of AgNPs and PGRs on proliferation of callus, MS medium complemented including 2 mg.L^{-1} 2,4-D was selected. The callus was cultured on Murashige and Skooge medium containing only AgNPs or combined with 2,4-D. AgNPs stock solution was prepared following the protocol of Rahman, Qureshi (16). Then the sterilized concentrations (20 and 40 PPM) of AgNPs were tested.

3.4. Fatty Acids Recognition Via GLC-Analysis Technique

Separated compounds of fatty acids were recognized in the laboratory of Dept. of Environment and Water/ Ministry of Science and Technology by Gas-Liquid chromatography (shimanezo, Japanese, 2010) utilizing ionized spark sensor and utilizing poetic column type SE-30 , together with 30m length via

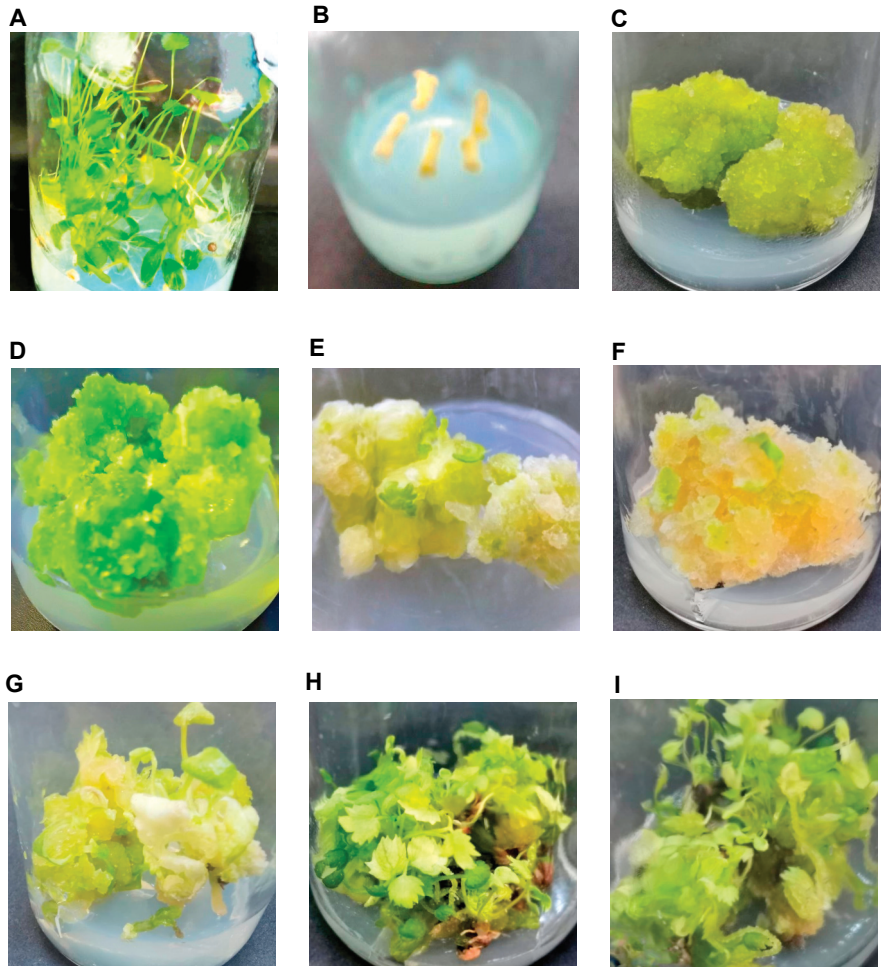

Figure 1. **Pictorial presentation of *in vitro* cultures in *Foeniculum vulgare* L. A)** 5-weeks old axenic seedling **B)** Sterile inoculated explant on culture medium showed callus induction. **C)** Callus production on MS medium supplemented by 2,4-D (2mg.L^{-1}). **D)** Callus proliferated on MS media with AgNPs 20ppm. **E)** Callus proliferation on MS medium conatining AgNPs 40ppm. **F)** Callus proliferated on MS media with 2,4-D (2mg.L^{-1}) & AgNPs 20ppm. **G)** Proliferated callus on MS medium supplemented by 2,4-D (2mg.L^{-1}) & AgNPs 40ppm. **H)** Shoot development of *Foeniculum vulgare* since callus grown-up on MS medium including AgNPs 20ppm. **I)** Shoot development of *Foeniculum vulgare* since callus grown-up on MS medium including 2,4-D (2mg.L^{-1}) & AgNPs 20ppm.

diverse diameters ranging between 0.25 and 0.5 mm. The temperatures were in the introduction space and sensor ranged between 280 °C to 330 °C, whereas the temperatures of the column starting at 120 to 280 °C at ratio of 8/min. utilized inactive nitrogen gas-like transporter at a pressure ratio of 100 kp. Fatty acid determination was carried out operating gas chromatography in accordance with the method of Abidi, List (17) and Rayford, Thomas (18). Starting with powdered samples, 200 mg were added to screw-cap tubes having 50 mL size. For aech indvidul container 5.0 mL of sodium methoxide with conc. 1% were suplemented, Prepared from 25% sodium methoxide solution (Grade HPLC/UV). 1:25 dilution of methanol (HPLC/UV). Tubes homogenized in vortex tubes stirrer (15 min intervals). Extracted for 1 hour, until esterification is complete. After 1 hour, add 1.0 mL of aqueous degreaser acetic acid (10% hydrochloric acid) and 10 mL of heptane Grade HPLC/UV. Homogenized in vortex tube stirrer. Transfer 2.0 mL of upper heptane (fatty acid) layer to GC vials.

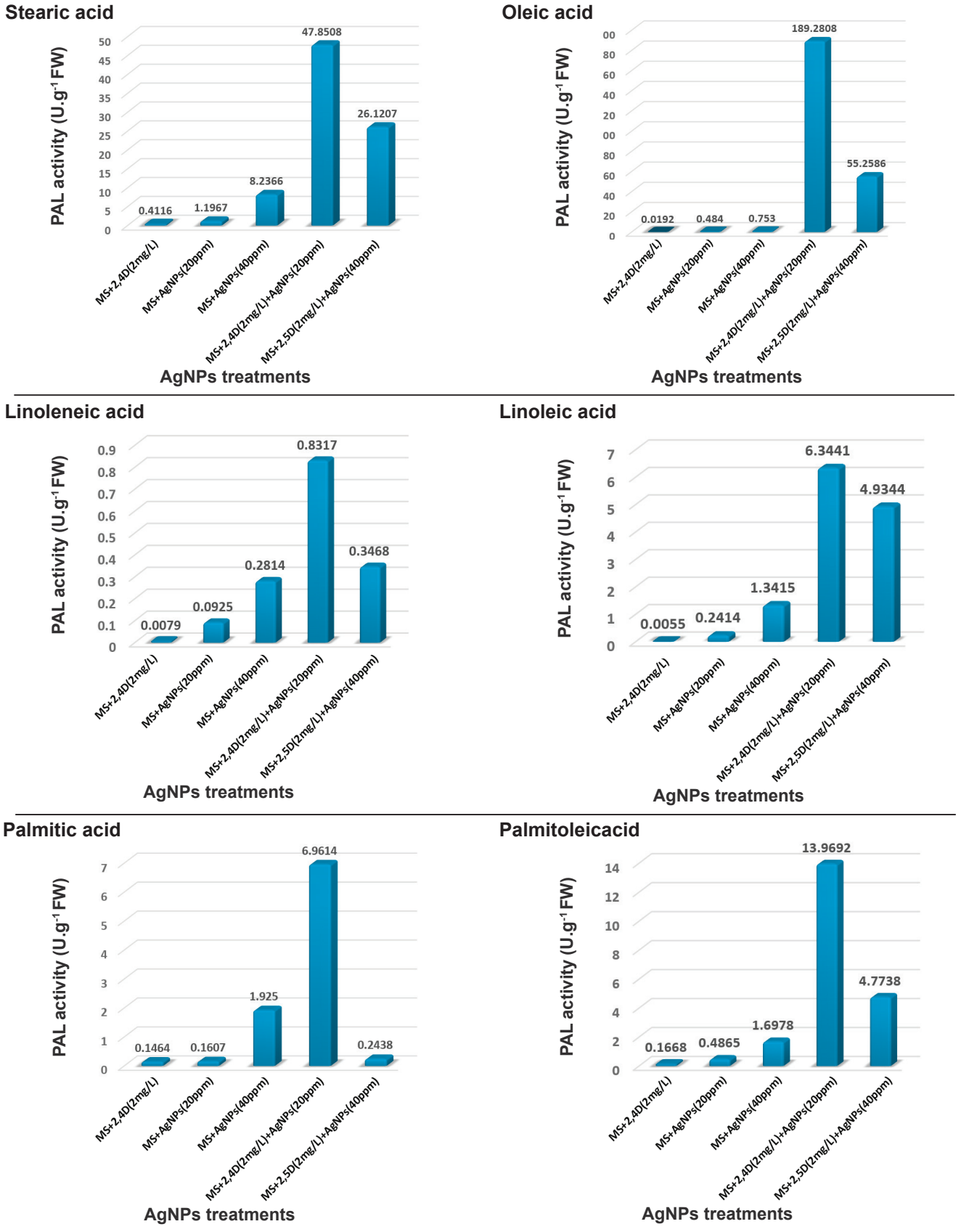

Figure 2. **Assessment of the fatty acid in the callus culture of *Foeniculum vulgare* L.** was determined in result to various concentrations of AgNPs additions. Statistics denote the mean significances of triplicates by ± standard error: stearic; oleic; linoleic; linolenic; palmitic and palmitoleic.

3.5. Determination of Vitamins Using HPLC-UV Device
Vitamins were determined in the Dept. of Environment and Water/ Ministry of Science and Technology laboratories by utilizing HPLC device (sykamn of German origin with a velocity of 1.3 $mL.min^{-1}$). The mobile phase contains Methanol: Distal Water and Formic acid at the ratios (70:25:5) using (18-ODS) column with 25 cm * 4.6 mm dimensions, the responses were noticed at 280 nm via UV spectrophotometer. The gained supernatants from total particular callus tissue were exhausted to quantify vitamins (E, D3, A, K, B2, and B6) that were defined adopting the modified methods of Gupta, Jain (19)

3.6. Statistical Analysis
The results have been expreesed as mean SD values,which were considered statistically significant at P value 0.01. Dunkin test was used to compare among all different groups, whereas the mean holding at least one common letter is not significant, while the mean holding completely different letters is considered to be significantly different .

4. Results

4.1. Impacts of AgNPs in Callus Formation and Proliferation in F. Vulgare L.
Usages of AgNPs significantly influenced growth of callus, proliferation and synthesis of valuable fatty acids and vitamin compounds. Independently the several concentrations of AgNPs did not significantly influence proliferation of callus. Compared with the control group MS+ 2, 4-D (2 $mg.L^{-1}$) by which 1.065 $g.L^{-1}$ fresh wieght of the callus biomass was recorded, where the maximum value (3.116 $g.L^{-1}$ FW) was observed at a higher dose (MS+AgNPs (20PPM) (. Established Calli were fragile and its colour is yellow-brownish (**Tables 2,3**). Further, Application in mishmash with 2, 4-D (2 $mg.L^{-1}$) + AgNPs (20PPM), callus biomass was significantaly enhanced. Maximum FW (7.32 $g.L^{-1}$) in total biomass accumulation of callus (**Table 1, Fig.1**), callus was charecctrized by its green colour and compact (**Table 3, Fig.1**). In the current paper, the maximuum amount of AgNPs (40ppm) beside an ideal value(20PPM) combined with 2, 4-D (2 $mg.L^{-1}$). The results has been a significant decrease in the biomass of callus, **Table 2**.

4.2. Impacts of AgNPs and PGRs on Percentage (%) of Fatty Acids in Callus Cultures of Foeniculum vulgare L.
Fatty acids (stearic, oleic, linoleic, linolenic, Palmitic, and palmitoleic) were determined, and it was found that the concentration increased by adding AgNPs. The Oleic acid was the fatty acid with the maximum percentage in oil (89.2808 %), next Stearic acid (47.851 %) then Palmitoleic acid (13.969 %) for callus cultured on MS + 2,4-D (2$mg.L^{-1}$) + AgNPs (20ppm) (**Fig. 2**), while the lowest value on callus cultured on MS + 2,4-D (2$mg.L^{-1}$) which were 0.4116, 0.0192, 0.0055, 0.0079, 0.1464, and 0.1668 For stearic, oleic, linoleic, linolenic, palmitoleic and palmitoleic respectively. In calli of *Foeniculum vulgare* L. induced by leaves tissues (**Fig. 2**).

4.3. Impacts of AgNPs and PGRs on Vitamins (E, D3, A, K, B1, B6) in Callus Cultures of Foeniculum Vulgare L.
Amongst the whole directed treatments, AgNPs at the intensity of 40 PPM devoid of PGRs caused in superior measures of vitamins E, D3, A, K, B1 and B6 (29.011, 1.861, 18.793, 1.959,1.097 and 1.245 $U.mg^{-1}$) within callus cultures (**Fig. 3**). No significant elevation within actions of vitamins was noticed within callus cultures launched by the influences of AgNPs in mixture with PGRs. Comparing with control treatment, by superior quantities of AgNPs (20 ppm) in mixture plus PGRs (2 $mg.L^{-1}$ 2, 4-D) which were 39.837, 2.683, 27.198, 3.885, 1.1904 & 1.581 $U.mg^{-1}$, but the lowest values obtained by MS + 2,4 -D (2 $m.gL^{-1}$), were (3.085, 0.164, 0.589, 0.219, 0.002, 0.365 $U.mg^{-1}$) (**Fig. 3**), respectively.

5. Discussion
According to dimension and intensity, the relation-ship of nanomaterials with plant cells effects a lot of physiological and progressive modifications in plant cellular development (20). Likewise, *in vitro* supplement of AgNPs is stated in the optimistic function of AgNPs in induction of callus and development in *Caralluma tuberculata* (21). Callus intiation was noticed within MS media through the special effects of AgNPs (60 PPM) in combo with 0.5 $mg.L^{-1}$ 2, 4-D + 3.0 $mg.L^{-1}$ BA.

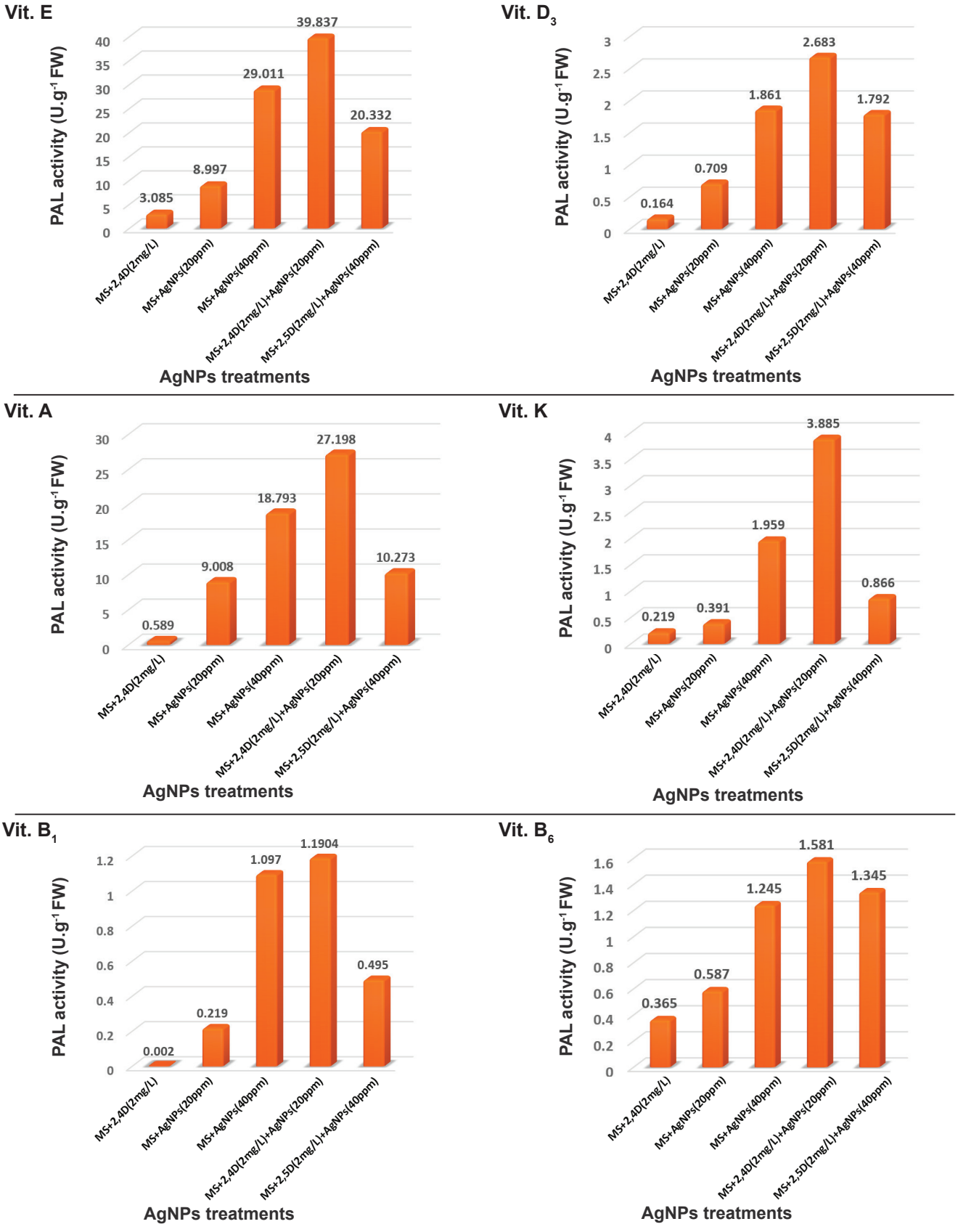

Figure 3. **Assessment of the vitamins (E, D3, A, K, B1, B6) within callus cultures of *Foeniculum vulgare* L. determined in result to various concentrations of AgNPs additions.** Statistics denote the mean significances of triplicates by ± standard error.

On the other hand, opposite to our results, various culture characters in the circumstances of the callus shape and color were observed in the cultures of *Caralluma tuberculata* complemented with AgNPs. In alternative research, distinctive intensities of AgNPs displayed pointedly optimistic results on the development of shoot & root in *Randia aculeata* L. (22). The alteration to yellow-brownish is the feature that denotes the foundation of AgNPs and which is because of the surface plasmon resonance (SPR), that is a size- conditional property of NPs (23). However, the strict method of the action of AgNPs in plant cell development is still not recognized. This could mean that by modulating the cell wall , AgNPs are able to increase plant cells acceptance of nutrients and water from culture media.

Parallel conclusions were established for lipid profile found from cell suspension of physic nut which was diverse from the profile of seed oil; though, there was an unlimited difference in the lipid profile between distinctive inhabitants of physic nut. In addition, these cell suspensions remain problematic,. quantitatively and qualitatively, to create a report for oil constructed from cell suspension (24, 25). The lipid profile gained from oil of callus was unlike the seed oil profile. There are numerous changes in the oil yielded in callus, for instance the existence of fatty acids (lauric, capric, pentadecenoic, & pentadecylic) that are not existing in oil of seed. The oil of callus yielded palmitic acid, myristic acid, and oleic acid in higher quantities than oil of seed. The oil of seed offers greater quantities of linoleic acid and stearic acid than oil of callus (26). Genetic propagation aims to enhance oleic acid, and to decrease linolenic and linoleic acids, which is significant since they participate in rising oil stability, avoiding oxidation and manufacturing of trans-fatty acids (27). The records of GC technique revealed that the significance of the entire fatty acids was raised in *N. oculata* cells managed with 25 $mg.L^{-1}$ AgNPs (28). Numerous vitamins have great antioxidant capacity, involving water-soluble (vitamins C and B complex) also,lipid-soluble (vitamins A, K and E). Plants have an extensive range of vitamins that are important not only for person metabolism but to plants as well, for their redox chemistry and function as cofactors (29). Vitamins in foods can be improved either by optimizing the growth circumstances, standard plant breeding or via the usege of transgenic systems, a technique called bio-fortification (30). Additional significant finding is that sensitivity to photo oxidative stress in vitamin B6 lacking Arabidopsis plants are increased (31). The optimistic influences of AgNPs on plants have been testified. Savithramma, Ankanna (32) elucidated the improved seed germination and the seedling development of *Boswellia ovaliofoliolata* tree benefiting biologically synthesized AgNPs. Enhanced *Brassica juncea* shoot, root length and leaf area, plus corn and common bean were registered consuming AgNPs. Treated plants also showed improvement in biochemical contents involving chlorophyll pigment, carbohydrate and also protein, and actions of antioxidant enzymes (33). AgNPs also alter quantities of microRNA expression that regulate numerous physiological, morphological and metabolic processes within plants.

6. Conclusions

NPs play a great role in motivating photosynthetic vilocity , pigmentation in addition to enzyme activity which might be used to enhance crop yield. *In vitro,* AgNPs in combination with PGRs resulted in callus proliferation. However, a single application of AgNPs led to the development synthesis of higher quantities of various fatty acids and vitamin metabolites. This research include the first sensible use of nanotechnology in the biodevelopment of the cultures of plant callus. However, in order to understand the molecular mechanism of these nanoparticles in cell development process and secondary metabolism, further research is needed. In addition, the use of plant cell cultures helps to obtain uniform extracts and enables more control over AgNPs synthesis during all seasens of the. In order to face the next health challenges, it must also understand their movement and chemistry.

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Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author

upon reasonable request.

Statements and Declarations

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Competing Interests

The authors have no financial interest.

Author's Contributions

All authors contributed to data collecting. The first draft of the manuscript was prepared by Raghad Mohammed Abdullah, Hikmat Mustafa Masyab, Anwaar Fakhre AL-Taee and Safaa M. Bilal All authors read and approved the final manuscript.

Consent to participate

Not applicable.

Consent to publish

Not applicable

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