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Fabrication and Characterization for Phosphate Nanofertilizer through Polymer Coating Technique

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K E Y W O R D S

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A B S T R A C T

Many organic and inorganic materials were used to enhance plant growth and soil fertility, in the present study phosphate and polyvinyl alcohol (PVA) was used to prepare nanocapsules fertilizer by using Emulsification Solvent Diffusion Method (ESDM), nanocapsules of phosphate and polymer provide long-term sustained-release preparations, ensure long existence of fertilizer in attach with the plant. Particle size distribution was evaluated in addition to particle shapes were characterized by Transmission Electron Microscope (TEM), the activity of Nano-fertilizer was studied on Nigella sativa production and its extracts activity. The emulsification solvent diffusion method (ESDM) provided a good yield of P-nanofertilizer, nanoparticles size distribution ranged 40-150nm with particle size range 74.23nm

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1. Introduction

Excessive use of pesticides and fertilizers causes accumulation of such toxic materials in soil and environment, leads to environmental degradation and decreasing agricultural production, practically this will leads to more unfertilized lands, high cost reclamation and absence of food security [1-4]. Nanotechnology provides a good ways to scale down the over use of fertilizers and enhances sustainable agricultural by producing new generation of low cost, and low environmental potential. Nanofertilizers have high surface area, fast to diffusion and have ability to raise soil fertilization and support the plant growth [5, 6]. Phosphorus (P) is an important essential nutrient in plants, involved in several key plant functions, like cell proliferation, energy transfer, photosynthesis, forming of seeds and root [7, 8]. In medicinal plants phosphorous can promote the growth rate as well as increase the number and concentration of active materials [9, 10]. Calcareous soils in most parts of Iraq minimize the concentration of phosphorus availability to plants. Provides such type of soil with P- nanofertilizer can support sustainable delivery, constant of concentration and high availability of (P) element. Innovation of spherical nanoparticles from polymer and fertilizer provides a good controlling system, save the nutrients from leaching and therefore improves fertilizer use efficiency [11, 12]. Gradual release can be achieved by coating with many materials like plaster of Paris, polymers, Chitosan [6, 13]. In the present study Poly vinyl alcohol (PVA) was used to prepare P- nanofertilizer because of their unique properties like un-toxic and safe in use, water solubility, favorable physical and chemical characteristics in addition to their ability in provide controlled drug and/or fertilizers delivery systems [3, 14, 15].

2. Materials and Methods

I. Seeds of N. sativa

Seeds of N. sativa were supplied from local markets; more examinations were carried out in Seed Inspection and Certification Department (SICD), Baghdad/Iraq.

II. Preparation of P- nanofertilizer

P- nanofertilizer was prepared under laboratory conditions using an Emulsification-Solvent Diffusion Method (ESDM) with minor modifications [16]. Briefly, 50 mg of PVA was dissolved in 5 ml of dicholoromethane with continuous mixing for 12 hrs at room temperature to get homogenous mixture. 5 mg in 25 mL of the Calcium phosphate, Ca (H2PO4)2•H2O solution was dropwise added to the above solution with continuously stirring. The resulted mixture exposed to sonication for 2 minutes to generate S/O primary emulsion. This mixture was emulsified with an aqueous phase of 20 ml of PVA (1% w/v) to form S/O/W emulsion by rotating in magnetic stirrer (REMI, India) at 400 rpm. The organic solvent was evaporated by rotary evaporator (Heidolph, Germany) at 50°C. The P-nanofertilizer was harvested by centrifugation at 10000g for 30 min at 4°C as shown in Figure 1.

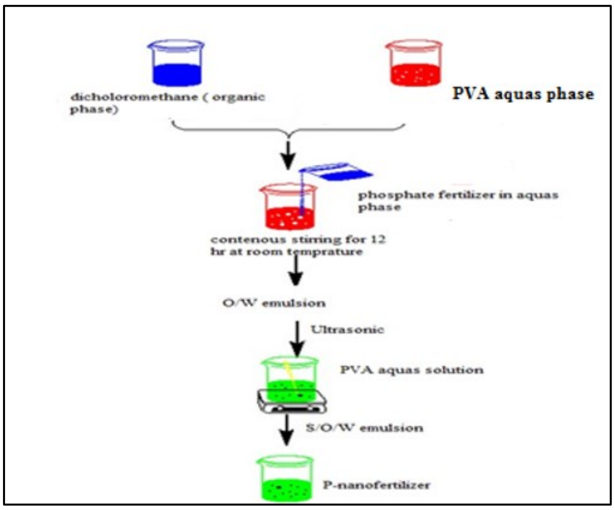


Figure 1: Diagram of experiment Emulsification Solvent Diffusion Method (ESDM)

III. Characterization of P- nanofertilizer

The zeta potential using Brookhaven Instruments Zeta Plus Potential Analyzer was applied to measure particle size distribution of synthesized P- nanofertilizer, with operating conditions of pH (3.0-12.0) at room temperature, 35 mV and 640 nm wavelength. While the morphological shape and size of P- nanofertilizers were examined using a transmission electron microscope (TEM-Philips Electronic Instruments, CM10 pw6020 Philips, Germany), by putting a drop of prepared P- nanofertilizer on TEM carbon coated copper grid, then all samples were dried on room temperature for TEM analyzing, while sizes of P- nanofertilizers were measured from the TEM images using an Image-Pro Plus 4.5 software. The final size was an average for five replicates.

IV. P- nanofertilizer effects on N. sativa

P- Nanofertilizer effects were studied on *N. sativa* under controlled lab conditions of 12 hours darkness and light consecutively at 25°C temperature. The experiments conducted in plastic germination pots 35cm ×25cm diameter with flat and pricket base. These containers filled with mixed soil collected from University of Technology gardens in plastic bags. 25 seeds of N. sativa were implanted in each pot, some parameters were measured like germination rate, dry weight, wet weight, and 100 seed weight.

Seeds numbers for each plant were determined according to type of treatment (non-fertilizer, recommended rate of imitative fertilizer 2g/kg, recommended rate of P- nanofertilizer 1g/kg)

V. Effects of P- nanofertilizer on Active Oil Contents from N. sativa Seeds

Active oil contents from seeds of N. sativa cultivated in 2g/kg of conventional P- fertilizer and 1g/kg P-nanofertilizer were estimated by Gas Chromatography Mass Spectroscopy (GC-MS, Shimadzu, Japan) by measuring retention time (RT) and band area for standard and samples, with the following conditions, FDI Detector, Col. Used (DP5.525.050), Initial Temperature (170ºC), Carrier Gas (N2), Rate (10 ºC /min), Initial Time (0 min), Pressure (100 Kpa), Final Time (10 min), Final Temperature (270 ºC), Total Flow Rate (81.4ml /min), Injector Temperature (280 ºC).

3. Results and Dissections

I. Characterization of P- nanofertilizer

Through synthesis process of P-nanofertilizer, the (P-PVA) solution transform from clear to turbid appearance, this conversion is evidence to formation of P-nanofertilizer. This formation occurs by molecular joining between PVA and phosphate molecules during the polymerization. Figure 2 shows a Transmission Electron Microscopy (TEM) image of the P-nanofertilizer, the nanoparticles appeared as granular shaped with harmonic size distribution, with mean diameter of the P-nanofertilizer (in the dry phase) up to 74.23 nm.
Figure 3 shows the zeta potential as a function of the pH for the P-nanofertilizer. The deference of the zeta potential with the pH values is a result of the changes on the surface charge of nanoparticles. The positive values indicate that cationic characteristics of PVA in the pH range of 2 to 6. The isoelectric point of the system is at pH = 6, where the positive and negative charges are equal. At a pH more than 6 negative zeta potential is appeared. This indicates that the outer surfaces of P-nanofertilizer charged negatively. The double electrical layer of the P-nanofertilizer causes relative stability within the colloidal distribution, while any electrolytes addition to a colloidal dispersion will cause inhibition the diffuse double layer and reduces the zeta potential [15].

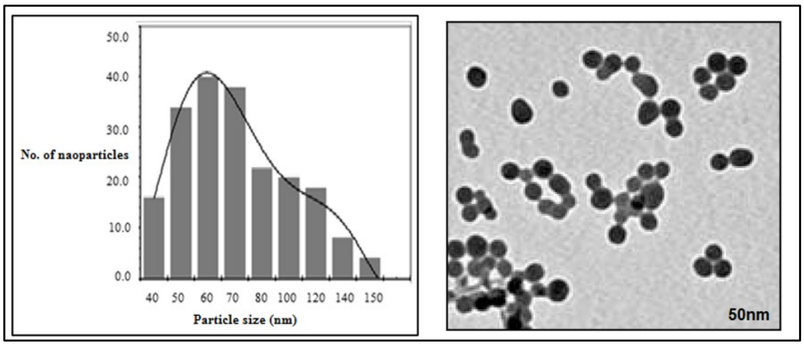


Figure 2: TEM image of P- nanofertilizer particle size distribution

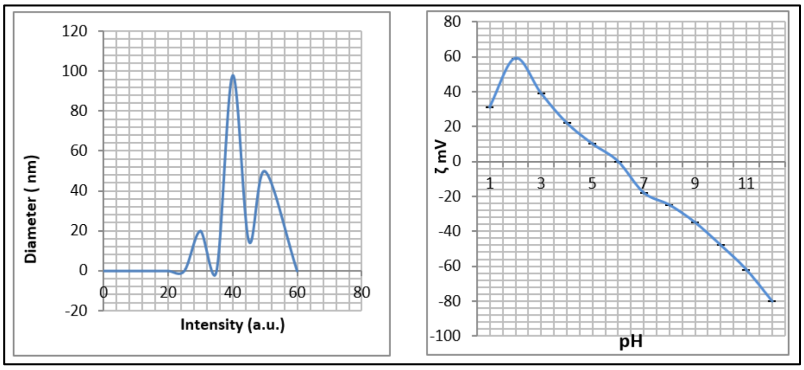


Figure 3: Particle size distributions (nm) and Zeta potential (mV) for P-nanofertilizers

II. P- nanofertilizer Effects on N. sativa

Table 1 showed that the treated N. sativa plants with the recommended concentration of P-nanofertilizers (1g/kg) gave significant differences from the other treats at p≤0.05, like germination percent (GR) (91%) at P-nanofertilizers treated while 63% at conventional P-fertilizer (2g/kg), however, speed germination (SG) was (5.5) seed/day in imitative fertilizer and reach up to (6.8) seed/day for P-nanofertilizers, other parameters like weight of 100 seeds, wet weight (WW) and dry weight (DW) all of these indicators achieved significant increasing when plants treated with recommended concentration of P-nanofertilizer, as shown in Figure 4. Also analysis of variance showed that the effect of P-nanofertilizer on oil percent (OP) was significant. The maximum of oil percent (32.6) % the minimum of oil percent (17.71) % of control treatment. Improvement in germination and growth rates, may be due to an improvement in enzyme content such as dehydrogenase, esterase, alkaline phosphatase, acid phosphatase, phytase [17-19]. The improvement of enzymes content promote the presence of high population of microorganisms in rhizosphere causes more P intake and mobilization [20].

Table 1: Some biological features for N. sativa seeds treated with conventional and P- nanofertilizers

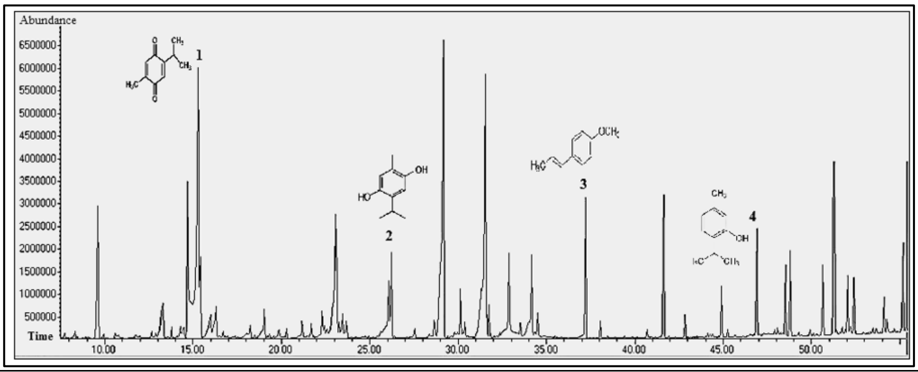
Treatment	GR%	SG (seed/day)	weight of 100 seeds (mg)	WW (gm/plant)	DW (gm/plant)	OP%
Control	55a	3.6a	188.58a	5.67a	3.23a	17.71a
P-fertilizer Conventional 2g/kg	63b	5.5b	225.73b	8.13b	4.18b	28.54b
P- nanofertilizer 1g/kg	91c	6.8c	435.56c	11.89c	6.52c	32.6c

Different letters refer to significantly from each other at p≤0.05, GR, germination rate , SG, speed of germination, WW, wet weight, DW, dry weight, OP, oil percentage.

III. GC-MS Analyses

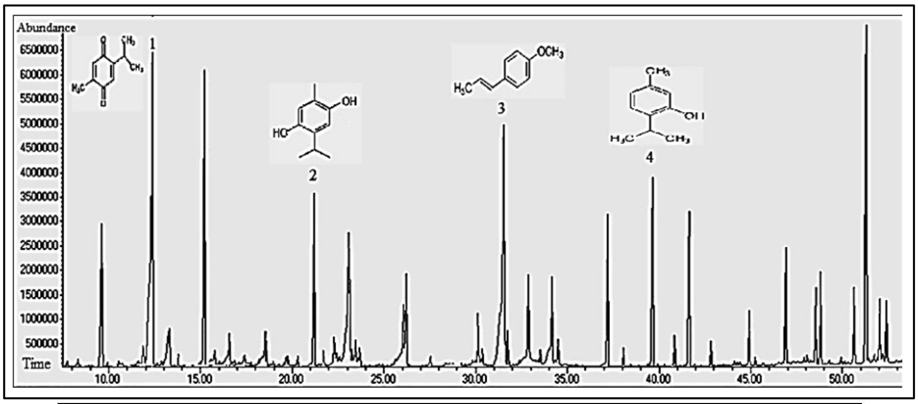
The N. sativa contains many secondary metabolites with potential therapeutic effects, these medicinal components concentrated in the seeds more than other parts of plants [21], the qualitative and quantitative variability of these compounds depends on several factors such as plant age, plant part, availability or unavailability of essential elements such as nutrients, temperature, water, light intensity. In this study tried to determine the effect of the P-nano fertilizer on concentrations of some known therapeutic components in N. sativa seeds by GC-MS analysis. The findings, summarized in Figure 5, refer to the abundance of four detected compounds Thymoquinone, Thymohydroquinone, Tras- anethole and Thymol [22, 23], and can be noticed increasing in concentration of all compounds in presence of conventional P-fertilizer. These concentrations of four compounds increased with recommended concentration of P-nanofertilizer as shown in Figure (6). The phosphate is most important energy compounds in the cell supports the biological processes within the plant by increasing photosynthesis, chlorophyll concentration and dry mass. The presence of P- nanofertilizer ensure high availability of (P) around plant root during all growth period, these lead to strengthen the biological process within the treated plants and causes increasing germination rate, wet weight, dry weight, oil content. These are appeared in GC-MS analyses curves for each treatment as shown in Figure 6.

Figure 4: (A) N. sativa height after 52 day of treated with 2g/kg of P- fertilizer (B*) N. sativa* height after 52 day of treated with 1g/kg of P- nanofertilizer



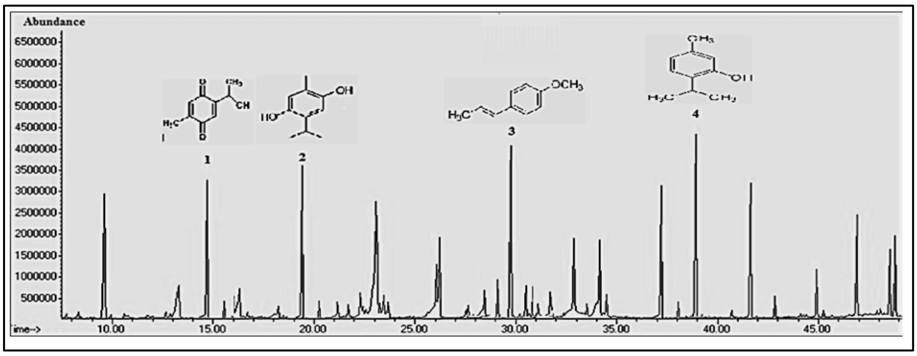
Active compound	Thymoquinone	Thymohydroquinone	Trans-anethole	Thymol
RT/min	15.23	26.33	37.01	47.66
Area	52481	33212	34614	42727

Figure 5: Gas chromatography analyses of *N. sativa* seeds extracted treated with conventional fertilize



Active compound	Thymoquinone	Thymohydroquinone	Trans-anethole	Thymol
RT/min	11.90	22.17	32.70	39.48
Area	٦٤٨٥٠	34800	50629	43078

Figure 6: Gas chromatography analysis of *N. sativa* seeds extracted treated P- nanofertilizers



Active compound	Thymoquinone	Thymohydroquinone	Trans-anethole	Thymol
RT/min	12.67	21.98	29.87	38.56
Area	34791	38293	40886	41660

Figure 7: Gas chromatography analysis of N. sativa seeds extracted untreated

4. Conclusions

Uses of phosphate- nanofertilizer in the recommended concentration (1g/kg) enhances the bio-indicators of *N. sativa* by improving the growth rates and oil contents of seeds due to elevate phosphate efficiency by increasing phosphate uptake and phosphate mobilization around plant rhizosphere. Emulsification Solvent Diffusion Method (ESDM) as a preparation method gave a range of nanoparticles from 40-150 nm with average reach to 74.23nm.

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