



Novel Nano-Sorbents Modified QuEChERS combined with GC-MS/MS for determination of pesticide residues used against *Spodoptera littoralis* in food stuff

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Abstract

The role of pesticides in the management of the destructive pest, *Spodoptera littoralis* is an essential element. Chlorpyrifos and Cypermethrin are the most commonly used insecticides in our country. Insect resistance against such insecticides enforced cultivars to increase the recommended doses which increases the pesticide residues in our food. This study's objective was to create a new methodology to measure the residue of Chlorpyrifos and Cypermethrin in potato and tomato yields. Chlorpyrifos showed high tolerance ratio equal to 380-fold in the field strain of *S. littoralis* compared with laboratory strain, while Cypermethrin showed a tolerance ratio of 5.79-fold.

A proposed modification of QuEChERS method to ensure the effectiveness of using different sorbent nanoparticles in the purification of both Chlorpyrifos and Cypermethrin residues from co-extracts can result in falsely favorable findings in order to guarantee the security of the commercially tomato and potato yields. A new, quick and easy analytical technique was created to determine Chlorpyrifos and Cypermethrin insecticide residues Using GC-MS/MS (gas chromatography-tandem mass spectrometry). In this method, Octadecyl-modified silica (C18), graphitized carbon black (GCB) and primary secondary amine (PSA) were converted to their corresponding nanoparticles, for the first time, and used in the sample purification step.

Acetonitrile was used to extract the samples and measured using internal standards and matrix-matched calibration. With values of $r^2 > 0.99$, all pesticides demonstrated good linearity in their respective ranges. With RSDs of 0.2 percent -14.4 percent, the average recoveries of the pesticide-spiked samples varied from 70.0 percent to -112.2 percent. The modified QuEChERS approach proved reliable and effective when used with tomato and potato samples to identify pesticide residues at trace levels.

Keywords: Chlorpyrifos, Cypermethrin, PSA, C18, GCB Nanoparticles.

1- Introduction

The cotton leafworm, *Spodoptera littoralis* is the most serious, destructive, pest in Egypt which belongs to (Lepidoptera: Noctuidae) and attack cotton crop and vegetables like potatoes, tomatoes, and other crops [1], causing great destruction to the different parts of the plant by feeding on leaves, flower buds or fruiting points. Potentially it is also a pest of glasshouse crops and therefore regarded as a quarantine pest. In Egypt, tomatoes and potatoes are the most affected crops after cotton, rice, and wheat, the most representative and widely cultivated food crops in the world in terms of human consumption [2, 3]. On around 3% of Egypt's total planted land, tomatoes are grown in the three seasons of winter, summer, and fall. Tomato crop losses have been significant. In terms of both the cash value and overall

tonnage produced, potatoes are the second-most significant vegetable after tomatoes.

The role of pesticides in *S. littoralis* management is vital to control this pest [4]. Chlorpyrifos and Cypermethrin are the most commonly used insecticides, in our country, to manage such a pest [5, 6]. Nevertheless, pesticide residues are detrimental to human health [7]. Therefore, it is important to keep an eye on pesticide residues in food. There have been numerous sample preparation methods documented up to this point for figuring out pesticide residues in food. A well-known sample cleaning technique called QuEChERS (rapid, easy, cheap, effective, robust, and safe) is based on dispersive solid-phase extraction with certain sorbents. The common sorbents used were C18 as adsorbent Primary secondary amine (PSA), which is capable of eliminating polar organic acids, pigments,

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and sugars are present in the presence of a magnesium sulphate and sodium chloride ($MgSO_4 + NaCl$) mixture and graphitized carbon black (GCB), as responsible for removing pigments and steroids [8]. According to the European Standard (ILNAS-EN 15662:2018), the recommended sorbents are PSA as a clean-up sorbent and C18.

Matrix effects (MEs), defined as the impact of one or more undiscovered components in samples on the measurement of an analyte's concentration or mass, are the most fundamental issue when complex matrices are addressed. Although analytical instruments are available, the proper qualification and quantification of analytes during pesticide analysis may be impacted by matrix effects, which can provide false-positive or false-negative results [9].

In order to regain consumer trust and enhance their reputations, a number of significant food producers, exporters, and importers in the agriculture industry are currently establishing ambitious traceability and quality control programs [10] concerning agriculture affairs including trace insecticide residues.

In recent years an observed significant increase in the factors that are important related to pesticide traces and rising demand for its better evaluation in our food was realized (upgraded). Although the original QuEChERS method is widely used, some modifications were created to improve the method's performance for particular challenging analytes, commodities and even for a low level of residues [10]. The modification assumes increasing the surface area of the sorbents (PSA, GCB, C18) to increase their effectiveness and this can be achieved using nanotechnology techniques.

Nanotechnology is the design, characterization, manufacture, and use of atomic, molecular, and macromolecular scale structures and systems for precise control of size and form, resulting in structures and systems with at least one novel characteristic or property [11]. To obtain nanomaterials of desired sizes, shapes, and functionalities, in this study, we use the ball milling technique as a physical method [11].

PSA, GCB, and C18 nanoparticles as adsorbents for the QuEChERS technique, however, have not been reported on. Therefore, the purpose of this work was to create a modified QuEChERS using nanoparticles PSA, GCB, and C18 as adsorbents for reducing the matrix effect to increase the effectiveness of sorbents and for the determination of Chlorpyrifos and Cypermethrin in tomato (*Lycopersicon esculentum*) and potato (*Solanum melongena*) using UHPLC-MS/MS technique.

Experimental

Laboratory strain of *S. littoralis*:

Laboratory strain (L-strain) of the cotton leaf worm, *Spodoptera littoralis* was obtained from a laboratory

colony continuously reared free from insecticides in Cotton Research Department, Agriculture Research Center, Plant Protection Research Institute, Dokki, Egypt. In the incubator, insects were raised in controlled environments at a temperature of 26°C and relative humidity of 65% according to [12].

Field strain

Field strain (F-strain) of the cotton leaf worm, *S. littoralis* was obtained by collecting the egg masses from Qalyubia Governorate before treatments during the 2021 season. As egg masses with hatching larvae, samples were transported to the lab for one generation under identical laboratory conditions to the previously mentioned laboratory strain. All cultures and reared on castor bean leaves until larvae reached the 4th instar which was used for the present study.

Tested Insecticides:

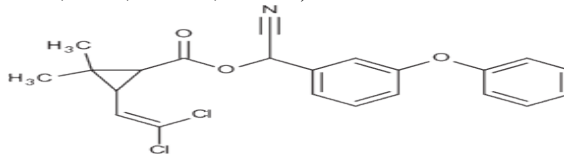
In this investigation, commercial pesticide formulations representing 2 major categories of insecticides used in cotton pest prevention programs (before Chlorpyrifos use is prohibited on food crops by the EPA, the limits for all commodities expire on February 28, 2022, and this final rule goes into effect on October 29, 2021) (EPA, 2021). These insecticides include organophosphates, Dursban H- 48% EC (Chlorpyrifos) obtained from Kafr El-Zayat Pesticides and Chemicals Co. and pyrethroids Cyper (Cypermethrin 10% EC) obtained from Shaanxi Sungen Road Bio-Science co.ltd. China.

EPA, Federal Register for Monday, August 30, 2021 (86 FR 48315) (FRL-5993-04-OSCPP) EPA-HQ-OPP-2021-0523; Chlorpyrifos; Tolerance Revocations, <https://www.regulations.gov/document/EPA-HQ-OPP-2021-0523-0001>

Synthetic pyrethroid compound:

a) Cypermethrin: (Alphathrin, 10% EC)

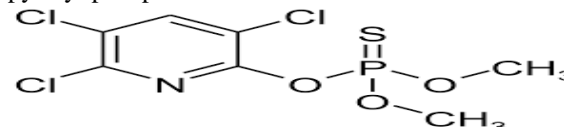
IUPAC Name: (RS)- α -cyano-3 phenoxybenzyl-(1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate (4 isomer pairs: cis-1, cis-2, trans-3, trans-4).



b) Organophosphorus compound:

Chlorpyrifos: (Dursban, 48% EC)

IUPAC Name: O, O-diethyl O - 3, 5, 6-trichloro -2-pyridyl phosphorothioate. The rate dose is 1L/faddan.



Insecticidal activity against *S. littoralis* larvae.

S. littoralis larvae in their fourth instar, from both the L and F strains, were subjected to bioassays to measure

the efficacy of the two insecticides. Using the commercial formulations, a variety of aqueous concentrations of each pesticide was created. The median lethal concentration (LC_{50}) values were calculated using the leaf-dipping method. Castor leaves were taken from untreated plants, cleaned, and dried. After being submerged for 30 seconds in each pesticide concentration, castor leaves were allowed to air-dry for one hour. The 25 recently molted 4th instar larvae of each strain were given the treated leaves to eat after starving for about 24 hours and maintained under $27\pm 2^\circ\text{C}$ and RH $65\%\pm 5$. Mortality percentages were recorded 24 h post-exposure. Each treatment (concentration), including control, was replicated four times.

Statistical analysis:

Using the Abbott formula, the corrected mortality was corrected [13]. Data were subjected to prohibit analysis as described by [14] through the use of the Ldp Line program (www.Ehabbakr Software/Ldp Line). By determining the resistance ratio (RR) from the comparison of the LC_{50} values of field and laboratory strains, the level of resistance was ascertained. Three levels of insect resistance were identified: low resistance (RR 1), moderate resistance (5 RR 10), and high resistance (RR > 10) [15].

QuEChERS application

Salts and buffer applying [16] Magnesium sulphate, sodium chloride, NaCitrate tribasic dehydrate anhydrous, and NaCitrate dibasic sesquihydrate are all ingredients in the QuE-Citrate Extraction tubes (55227-U) that are used to extract samples. A dispersive solid-phase extraction (d-SPE) 10 mL polypropylene centrifuge tubes containing 0.9 gm of magnesium sulfate were prepared. Supelclean Primary secondary amine SPE Bulk (52738-U) from Supelco, Graphitized Carbon Black Carbotrap, 20-40 mesh, a bottle of 10 gm (20287) and C18 from Supelco.

Active ingredients for Chlorpyrifos (CAS Number: 2921-88-2) and Cypermethrin (CAS Number: 52315-07-8) and aldrin (CAS Number: 309-00-2) pesticides were acquired from Dr. Ehrenstorfer-LGC GmbH (Augsburg, Germany) applying ISO-17034 for reference material with purity more than 95%. Acetonitrile HPLC-grade $\geq 99\%$, n-Hexane $\geq 99\%$, Acetone-HPLC grade $\geq 99.0\%$ were all from Merck. Class-A glassware was utilized for the volumetric flasks and pipettes, also calibrated micropipettes (Hirschmann Laborgerate) to prepare calibration with ranges (10-100, 100-100 μl) were used. Solvent dispenses of 10mL capacity (Hirschmann Laborgerate) for Acetonitrile. Stock solutions of 1000 $\mu\text{g/ml}$ for all pesticides were individually prepared and dissolved in Toluene deepfreeze and stored at -20°C , three intermediary

combinations were created at levels 10 $\mu\text{g/ml}$ then calibration mixtures at 10,50,100 and 500 ng/ml were made from serial dilutions and prepared in toluene of the working solution where aldrin as injection maintaining the standard at level 100 ng/mL in every calibration and the intermediate mixture kept at -4°C in the refrigerator.

2.2. Synthesis of C18, GCB and PSA nanoparticles

Commercially available adsorbents of Octadecyltrimethoxysilane (C18), Graphitized Carbon black (GCB) and Primary Secondary amine (PSA) were milled individually in 250 mL steel cells with 15 mm hardened steel balls that weigh 32 grammes in ambient air for varying intervals between 2 and 50 hours. The mechanical milling was done in a Retsch PM 400 horizontal oscillatory mill running at 25 Hz. Without further milling media, the materials that were already milled were utilized. Each cell included 10 g of the sample powder along with five balls for each adsorbent. In this experiment, there were two parallel cells employed (the total weight for the sample powder was 20 g). The characterization of these synthesized materials was done.

2.3. Characterization of C18, GCB and PSA nanoparticles

Transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FT-IR), and Brunauer-Emmett-Teller analyses were used to characterize the produced C18, GCB, and PSA nanoparticles (BET). The XRD measurements on powder samples were done using an ANalytical X'Pert PRO X-ray machine, X-ray source was Cu Ka radiation (45 kV, 30mA). The method of FT-IR was with KBr pellet, the FT-IR disk process. 1 mg sample (C18, GCB and PSA nanoparticles respectively) was ground each with 200 mg KBr for tablets, respectively. The percent of the sample to KBr was 0.5%. The wave numbers of FT-IR measurement range were controlled from 400 to 4,000 cm^{-1} . TEM pictures were captured using a 200 kV operated JEOL JEM 2000EX. A small quantity of the powders was mixed with a few milliliters of regular butanol in an ultrasonic bath and sonicated for 30 minutes to prepare the TEM specimens. A drop of this dispersion sample was then applied with an eye dropper to a copper grid covered in holey carbon film. Before evaluation, the samples were dried at room temperature in a vacuum oven. Before the picture was shot, the sample was scanned throughout all zones. The BET adsorption equation was used to calculate the sample's specific surface area. By using the Langmuir method, estimates of the samples' cumulative pore volume, cumulative pore volume, average pore diameter, and BJH desorption pore distribution curves were made [17].

Apparatus

For automated shaking of samples in both extraction and d-SPE, Geno/Grinder 2010- SPEX Sample Prep (UK) and Agilent benchtop GC-8890 with MS-7010B triple quad system and high-efficiency (HES-EI) ion source was used (Sigma, Germany 3-16KL). The sample was evaporated at 40 °C using a Heidolph rotary evaporator (Hei-VAP Core, Heidolph Instruments GmbH & CO. KG).

Sample preparation

Ten gm of homogenous samples of tomatoes and potatoes were used to apply QuEChERS method [16]. E7-citrate salts extraction module then dispersive solid-phase extraction follows (d-SPE) 10 mL polypropylene centrifuge tubes containing 0.9 gm of magnesium sulfate in addition to C18, PSA, GCB according to the following scheme:

For potato matrix, three replicates and a blank sample for each sorbent test were weighted in a 50 ml PP tube. Three replicates were spiked with the target pesticides at 0.05 mg/kg applying [16] E7-citrate salts extraction module, then the sample was centrifuged for 5 minutes for two minutes at 4500 rpm. The upper layer of acetonitrile was transferred to 10 ml PP tube containing 0.9 g of MgSO₄ and 0.05 gm C18 or PSA in bulk or nanoparticles. The same steps were followed for tomatoes in addition to GCB sorbent due to the tomato's pigments.

The d-SPE tubes were shaken for 30 seconds, centrifuged at 4500 rpm for 2 minutes, and then had 0.45 m syringe filter membranes applied after 2 ml of the acetonitrile layer had been placed into a 50 mL flask and nearly dried off at 40 °C.

The samples were applied to the GC-MSMS system for quantifications and compared to the results obtained via the application of normal particles.

GC-MSMS system conditions

In the split-less mode, where the inlet temperature was maintained at 250 °C, the injection volume was 1l. Separations were performed on the first column HP5 ms UI, which had the following specifications: capillary column, mass spectrometer, 70 cm of inert fused silica ID-0.15 mm, 30 m length, 0.25 mm internal diameter, and backflush union. Following injection, the column oven temperature was maintained at 70 °C for 1.3 minutes before ramping from 70 °C/min to 200 °C, ramping at 50 °C/min to 270 °C, and ramping at 50 °C/min to 310 °C, which was maintained for 6.3 minutes before a 2-minute post-run end-point backflush at 310 °C. The temperatures of the transfer line, quads, and ion source were 250 °C, 150 °C, and 300 °C, respectively. The standard production values for the helium quench gas and the 99.999

percent pure nitrogen for the collision gas were established at 1.5 mL per minute and 2.25 mL per minute, respectively. An MS system with MRM ion transitions and a retention time window for each chemical was given a multi-reaction mechanism. Compounds were only observed simultaneously when they transitioned from GC to MS, as the number of data points for each compound increased. Table 1 lists the MRM requirements, including the pesticide name, the retention time (RT Minutes), the qualifiers (Q2, Q3), the quantifier (Q1), and collision Energy (CE, ev)

Pesticides	RT	MRM Transitions and CE
Aldrin	6.627	263.0 -> 193.0 (25)
		293.0 -> 186.0 (30)
		298.0 -> 263.0 (8)
Chlorpyrifos	6.644	313.8 -> 258.0 (14)
		196.9 -> 168.9 (25)
		196.9 -> 107.0 (15)
Cypermethrin	10.202	162.9 -> 127.0 (5)
		181.1 -> 127.1 (35)
		181.1 -> 152.1 (25)

3. Results and discussion

Evaluation of insecticidal activity against *Spodoptera littoralis*

The LC₅₀ values and the calculated RR of used insecticides tested against the laboratory and field-collected *S. littoralis* strains were recorded in Table (1) Both tested compounds used in the present work caused variable toxicity levels against the 3rd larvae of *S. littoralis* for both laboratory and field strains.

Calculated LC₅₀ values were 0.5 and, 16.9 ppm for Chlorpyrifos and Cypermethrin against *S. littoralis* laboratory strain, while field strain recorded 190.3 and 100 ppm for Chlorpyrifos and Cypermethrin. Chlorpyrifos was more toxic to *S. littoralis* laboratory strain, while Cypermethrin showed more toxicity against the field strain. Concerning resistance ratio, data in Table (1) reflect the appearance of higher resistance formed in *S. littoralis* toward the organophosphate, Chlorpyrifos in the field, the resistance increased by 380-fold. Low RR value (5.79-fold) was recorded during application of Cypermethrin against *S. littoralis* field strain. The calculated resistant ratios proved the frequent application of such insecticides against *S. littoralis* in our fields. The elevation of insecticide resistance enforced cultivars to increase the used doses of insecticides which subsequently increase its residues in our food.

Table 1: Toxicological data of Chlorpyrifos and Cypermethrin insecticides against field and laboratory populations of *S. littoralis*

Insecticides	Strains	LC ₅₀ ppm (Lower- upper)	LC ₉₀ ppm. (Lower-Upper)	Slope ±SE		RR
Chlorpyrifos	Laboratory	0.55 (0.29-0.94)	6.36 (4.16-21.13)	1.2±0.09		-
	Field	190.3 (139.74-416.74)	1115.95 (1114.66-10988.4)	1.67±0.18		380 fold
Cypermethrin	Laboratory	16.97 (12.76-25.85)	45.16 (43.17-157.3)	3.01±0.3		-
	Field	100.0 (67.92-140.24)	400.69 (304.86-748.3)	2.15±0.15		5.79 fold

Table (2): The effect of using nano and bulk sorbents on, matrix effect %, recovery% and sensitivity during insecticidal residue extraction from potato

Pesticide	Chlorpyrifos				Cypermethrin			
	Nano size		Normal size		Nano size		Normal size	
Sorbent type	C18	PSA	C18	PSA	C18	PSA	C18	PSA
Mean recovery*	111	119	101	129	110	140	167	130
Matrix effect%*	-7	-19	-9	6	-29	-69	-50	12
Signal: noise (S/N)	74	19	∞	12.5	∞	∞	∞	∞

Table (3) The effect of nano and bulk sorbents on, matrix effect % , recovery% and sensitivity during analyzing pesticide residues in tomato matrices

Pesticide	Chlorpyrifos						Cypermethrin					
	Nano size			Normal size			Nano size			Normal size		
Sorbent name	C18	PSA	GCB	C18	PSA	GCB	C18	PSA	GCB	C18	PSA	GCB
Mean recovery%*	129	115	60	140	112	60	164	117	86	86	95	86
Matrix effect%*	-6	-21	-12	-26	-12	-12	-62	-63	-62	-74	-63	-62
Signal: noise (S/N)	88.4	39.5	10.3	29.9	27.5	11.8	∞	∞	∞	∞	∞	∞

Estimation of pesticide residues using QuEChERS - QuEChERS method

a-Characterization of nanoparticles C18, PSA and GCB sorbents

3.1. XRD Analysis

The pattern of C18 nanoparticles, Fig (1), exhibited a broad signal at (2θ) of 24.41° indicating the amorphous nature at the same position of the bulk C18 suggesting no change was recorded after the milling process. The XRD patterns of normal and nano PSA (Fig 1) showed a broad peak between 19° and 27° (2θ) characteristic of the amorphous nature of PSA and again did not show any change in the position or the width of the signal due to the milling process and formation of the nanoparticles. The peaks observed at 2θ values of 25° and 42.8° for the original graphitic carbon black and are assigned to the (002) and (101) diffraction planes of graphitic carbon, respectively [18].

These signals were slightly shifted to 26 and 43.10 for the nano graphitic carbon black with slightly broadening revealing the decrease in the particle size. The presence of a broad peak at 180 in both samples

indicates the presence of large amounts of amorphous carbon in the material [19].

3.2. FTIR spectra:

The comparative FTIR spectra of bulk C18 and C18 nanoparticles are shown in Fig 2. For Normal C18, the hydroxyl groups ν(O-H) stretching modes are attributed to the area between 3600 and 3000 cm⁻¹. The largest peak in silica, at 1104 cm⁻¹, is associated with the Si-O bond and is part of a typical region of peaks in the range of 1250 to 700 cm⁻¹ (Fig. 2). At 802 cm⁻¹, the ν(Si-O-Si) band's symmetric mode can be discovered. Alkyl groups can be seen in the area where the C-H molecule is stretching with bands at 2962 cm⁻¹ which are assigned to C-H asym for C18 and both bands at 2926 and 2854 cm⁻¹ assigned to CH₂ ν(C-H)_{asym} and CH₂ ν(C-H)_{sym}, respectively [20].

For nano C18, FTIR spectrum showed similar bands as the normal C18 suggesting that the functional groups did not change by decreasing the size to nanosized.

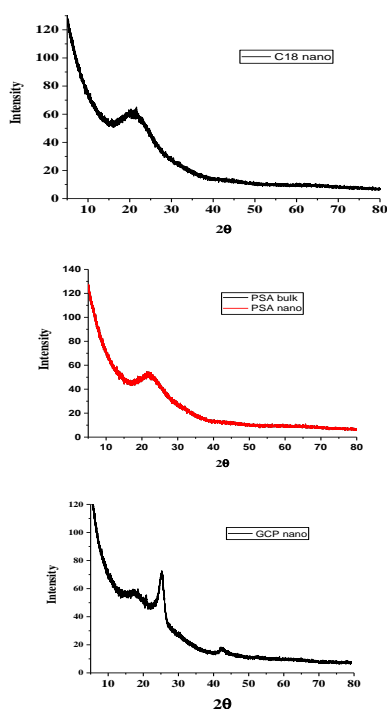


Fig 1: XRD of C18, PSA and GCB

The FT-IR spectra of commercial PSA and PSA nanoparticles are shown in Fig 2. The commercial PSA showed peaks at 2889 cm^{-1} and 2978 cm^{-1} respectively due to $-\text{CH}_2-$ vibrations, and absorption in the range of $3000\text{--}3700\text{ cm}^{-1}$ due to the vibration of $-\text{NH}$ and $-\text{NH}_2$ groups and $-\text{OH}$ on the surface of the silica. The distinct bands as shown at 1640 and 1466 cm^{-1} were due to bending of a secondary amine and asymmetric and bending of primary amine (NH_2) [21]. The strong bands at 1084 cm^{-1} and 797 cm^{-1} were characteristic of Si-O-Si anti-symmetric and symmetric stretching vibration for commercial PSA, respectively. As opposed to that, the FTIR spectrum of nano PSA showed characteristic bands similar to that of the commercial PSA with some shifts due to the grinding and formation of the nanoparticles. The comparative FTIR spectra of bulk and nanoparticles of graphitized carbon are shown in Fig (2). Normal Graphitized Carbon black exhibited stretching vibrations located at 3390 cm^{-1} , 1639 cm^{-1} and 1396 cm^{-1} showing the presence of $-\text{OH}$ groups [22] in GCBs. Both transmittance it was possible to attribute bands that were present at close to 2920 cm^{-1} and 2850 cm^{-1} to the C-H stretching vibration [23] and bands approximately at 1572 cm^{-1} for a C=C stretching vibration of graphene. On the other hand, the FTIR spectrum of GCB nanoparticles obtained exhibited a new band associated with the C-O vibration at 793 cm^{-1} appeared may be due to structural defects of the graphene through grinding.

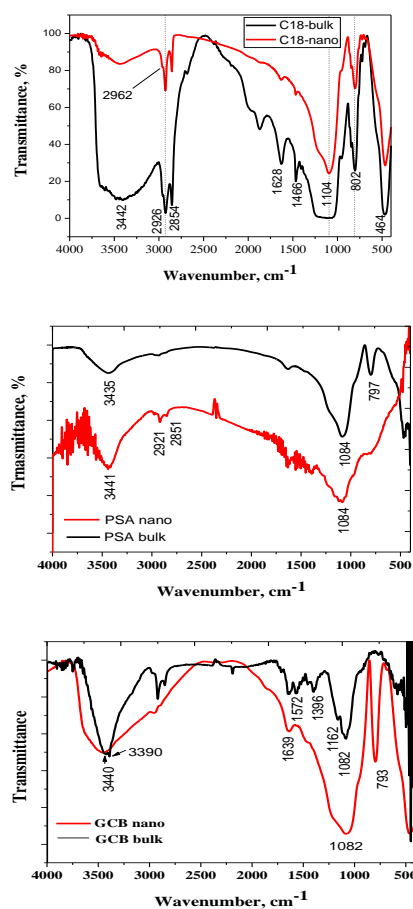


Fig 2: Commercial PSA and PSA Nanoparticles' FT-IR spectra

3.3 TEM characterization:

The morphology of the surface of the synthesized materials was characterized by TEM and the results are depicted in Fig (3 -a-g). It could be seen from Fig 3. a and b that the size of the normal C18 is reduced from 2 nm to around 50 nm after grinding. The average size is 2000 nm and 40 nm for C18, 220 nm and 30 nm for GCB and 200 nm and 30 for PSA. The TEM images of nanoparticles are monodispersed; almost all of them quasispheroidal shapes with uniform size and smooth surfaces. PSA NPs Small particles' incredibly small dimensions and high surface energies cause them to agglomerate into secondary particles.

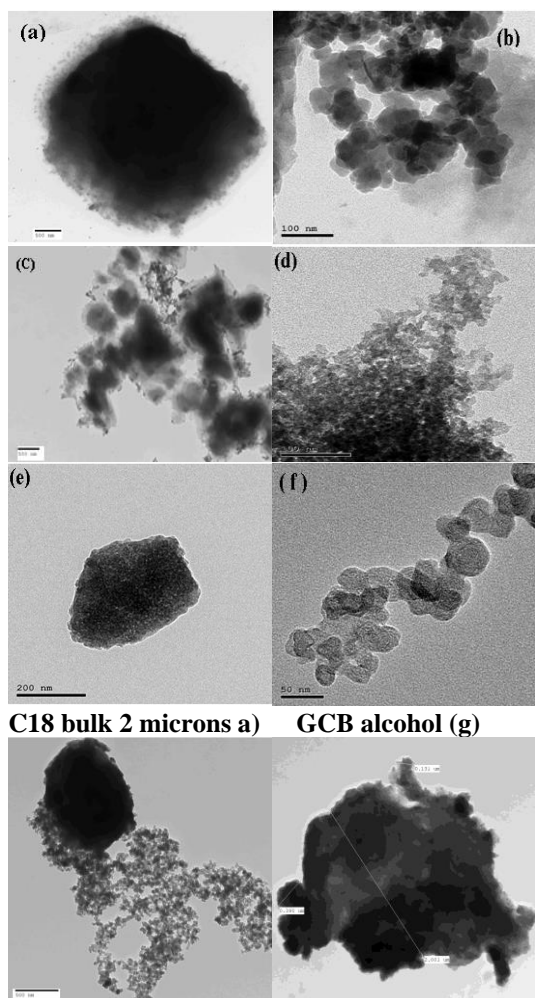


Fig 3: The TEM images of commercial PSA, C18 and GCB and nanoparticles of PSA, GCB and, C18

It is important to mention that when the normal sample of GCB was sonicated for 15 min in a sonication bath the material get dissociated to the nanosized as shown in Fig 3 (g).

4- Effect of using nano sorbents on matrix effect %, recovery% and sensitivity of selected pesticides in potatoes

During the determination of Cypermethrin in potatoes, using nanoparticles (NP) of C18, compared to normal size, decreased the matrix suppression effects due to the presence of sugar and starch in potatoes - 29 % vs -50% while had slight effect in Chlorpyrifos maximum -9%. Using NP-PSA compared to normal size, during extraction of Cypermethrin and Chlorpyrifos, proved to have no remarkable effect on reduction of co-extract matrix compared to the results of C18. The mean insecticide recovery for three replicates to demonstrate the effect of both nano and normal particles using QuEChERS extraction method showed reasonable recovery with small bias using C18 nanoparticles compared to normal particles for both Cypermethrin 110% and

Chlorpyrifos 101% where later was almost unaffected during the test. Applying the same extraction method using PSA it gives satisfactory results for only Chlorpyrifos either using nano 119 % or normal size 129% sorbent rather than for Cypermethrin. Also, S/N when using NP-C18 is 58% larger compared to normal C18 as clean-up sorbent indicating high sensitivity relative to Chlorpyrifos using nano particles while Cypermethrin could not predict the matrix effect due to non-significant changes observed also the data was supported with the injection of a blank sample of potatoes applying the same conditions and higher S/N using C18 NP indicates low co-extractive interferences compared with target compound. From figures of potatoes blank injection for C18 case, the noise inside the region where Cypermethrin and Chlorpyrifos eluted decreased sharply which decrease the effect on the background due to the co-extract matrix of potatoes during analysis that could interfere with the target analyte. From our findings, using C18 nanoparticles for extraction of both Cypermethrin and Chlorpyrifos from vegetables is recommended.

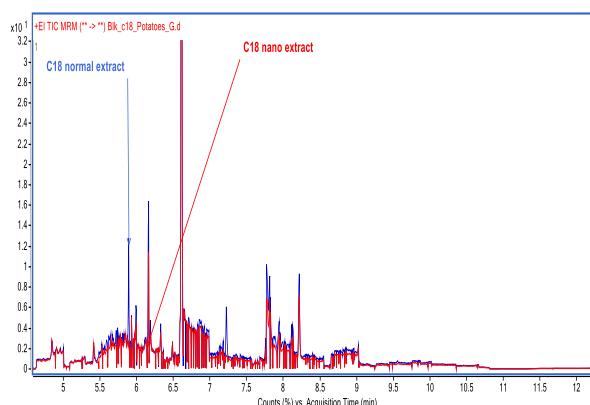


Fig 3: Total ion chromatogram overlay of (insecticide) potatoes blank using both nano and bulk C18 sorbent

Comparing the results of using C18, PSA and GCB either normal or nano-particles for removal of a carotenoid called lycopene pigment via investigation of standard addition of 50 ug/ml on the extract, the data obtained does not give clear justification because almost the same values, but after calculating S/N for a blank extract that was previously treated with different sorbents in both physical shapes, the data obtained gives a remarkable high value of S/N after using nanoparticles of PSA and verified after calculation of mean recovery at 0.05 ug/kg where it was 115% for Chlorpyrifos and 117% for Cypermethrin. From data of blank tomatoes treated with a different sorbet of two physical shapes, we can notice generally the counts of signal that refer to sensitivity were duplicated while using nanoparticles compared to normal particles (Fig 3). For PSA case, the noise inside the region after 7 minutes, during elution of Cypermethrin and

Chlorpyrifos, decreased sharply which minimized the effect on the background due to the co-extract matrix of tomato during analysis due to the interference of the co-extract tomato matrix with the target analyte. From our findings, we can recommend using PSA nanoparticles for the extraction of both Cypermethrin and Chlorpyrifos from tomatoes.

Experts from Farmworker Justice cautioned that EPA is still allowing some non-food uses of Chlorpyrifos, such as on golf courses, ornamental plants, and non-bearing fruit trees, even though the ban on the use of Chlorpyrifos on food crops will provide significant protections for environmental and farmworker advocates.

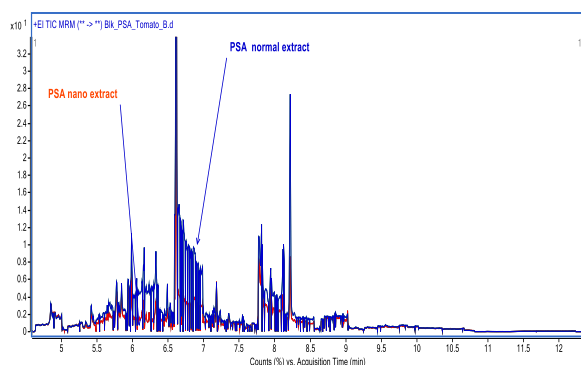


Fig 4: Total ion chromatogram overlay of tomatoes blank using both nano and bulk PSA sorbent

5- Conclusion

Results revealed the high doses used during the application of Chlorpyrifos and Cypermethrin during pest control management of *Spodoptera littoralis*.

A modified QuEChERS method was adopted to trace any elements or residues of insecticides in our food. Nano forms of C18, PSA and GCB were used for the first time, during the purification step. C18 nano form was found to decrease the potato matrix suppression effect during estimating Cypermethrin potato matrix suppression effect, but the slight effect was measured during estimation of Chlorpyrifos Nano-PSA did not show significant reduction of co-extract, matrix of potato treated with both insecticides, compared with C18 results. Concerning tomatoes, data gives a remarkable high value of S/N after using nanoparticles of PSA.

Results proved the high efficiency of nano forms of sorbents during measurements of residues using QuEChERS method.

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