RESEARCH PAPER

A New Method for Extracting and Measuring Methoxyfenozide Using ND/PAN-K10 SPE Using High Performance Liquid Chromatography

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ABSTRACT

A sensitive, economical, and miniaturized self-assembly needle tip nano-composite Polyaniline-K10 solid-phase microextraction (NDT/PAN-K10-SPNE) coupled with high-performance liquid chromatography UV detection was developed for rapid extraction removal and determination of methoxyfenozide in environmental water samples. The NDT/PAN-K10-SPE cartridge, assembled by packing 2.0 mg of PAN-K10 as sorbent into a needle tip, showed high adsorption capacity for methoxyfenozide owing to the large surface area and unique structure of PAN-K10. The factors that affected the extraction efficiency of PT-G-SPE, including sample volume, pH, sorbent amount, washing solvent and eluent solvent, were optimized. Good linearity for methoxyfenozide was obtained in a range of 0/01–100 µg mL−1 with the correlation coefficient of (R2) = 0.999. The recoveries of the methoxyfenozide at three spiked levels ranged from 84.72% to 100.03% with the relative standard deviations (RSD) of 3.78%. It is also noteworthy that the applied method is economical and has a good linear range, reproducibility, and high sensitivity.

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INTRODUCTION

Methoxyfenozide (MOF) [3-Methoxy-2-methylbenzoic acid 2-(3,5-dimethylbenzoyl)-2-(1,1-dimethyl ethyl) hydrazide. RH-2485] is a bisacylhydrazine insecticide first introduced by Rohm and Haas Co. (1996). MOF pesticides belong to the bisacylhydrazine group as a new group of insect growth regulators and a non-steroidal compound with accelerating insect molting properties. Bisacylhydrazines are an environmentally friendly type of insecticides that have a new mode of action and are less dangerous to non-target organisms than conventional insecticides. They also mimic the action of the natural insect molting hormone (ecdysone) and induce early molting and larval death by direct binding to ecdysone receptors. They are more effective against sugar beet armyworm (*Spodoptera exigua*), European corn borer (*Ostrinia nubilalis*), and potato tuber moth (*Phthorimaea operculella*) [1]. MOF is absorbed rapidly and moderately, metabolized extensively, excreted from bile in the body, and is less observed in urine. MOF is most toxic through skin and inhalation and is less poisonous orally. Due to the dangers and toxicity of MOF to human health, its residues in fruits and vegetables can be considered a danger to humans and the cause of specific diseases. Therefore, it is of paramount importance to identify and determine the amount of pesticides, such as MOF, which are consumed by spraying fruits and vegetables. Thus, it is important to provide methods that can eliminate these pollutants from the environment. In the present study, MOF was successfully extracted and measured by modeling the previously used technique, which is based on the SPE methods. Instead of using a pipette or a sampler tip, steel syringe tips were used in this method, and a polyanylene-K10 nanocomposite as

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an adsorbent was placed at the tip of the needle for use in extraction.

Nanocomposites are a special class of composites in which at least one of their components is nanoscale. Most nanocomposites contain little amounts (usually < 5% by weight) of layered mineral fillers or single-wall carbon nanotubes.

Montmorillonite (MMT) [chemical formula: $nH_2O–(Na,Ca)(Al, Mg)_6(Si_4O_{10})_3(OH)_6$] is a very soft aluminosilicate mineral, which is usually formed as microscopic crystals and is used more than other clay soils in the manufacture of nanocomposites. MMT has a series of very ideal properties all of which together are not found in any other natural clay. For example, this substance is very broad and strongly ionic. The wideness of MMT means that each particle has a relatively large surface area, leading to a stronger interaction of the particle with the resin matrix. MMT is enclosed in an ionic network. Polar compounds, such as MMT, mix with other polar materials more easily with relatively high intensity. Some resins, such as nylon, are completely polar. Therefore, clay soils (e.g. MMT) strongly mix with nylon polymers. Accordingly, MMT hardly interacts with non-polar resins, which are mostly commercial resins [2, 3].

MMT clay has a layered structure consisting of octahedral alumina layers sandwiched with two tetrahedral silicate layers. The thickness of the layer is about 1 nm and the lateral dimensions of these layers range from 30 nm to several microns [4]. Montmorillonite layers have negative charges due to the fusion substitution effect and are neutralized by mineral cations such as Na^+ and Ca^{2+}. These mineral cations are interchangeable, making MMT an effective adsorbent for various cationic contaminants such as heavy metals, pigments, surfactants, and monomers. After the adsorption of organic cations, the interlayer space of MMT, which has hydrophilic properties, turns into a space with organophilic properties. The resulting MMT, known as organic clay, is employed as an effective adsorbent for hydrophobic organic pollutants [5-9].

In this work, a conducting polymer, polyaniline (PANI), was combined as a guest with layered MMT clay, and the application of this nanocomposite as an adsorbent was investigated in the PT/SPE method. In SPE methods, it is of great import to use adsorbents with a large surface area since they play an essential role in analyte extraction. Accordingly, PANI stabilization at the surface of MMT K10

leads to the preparation of an adsorbent with both a high removal efficiency and a large surface area, thereby improving the adsorption properties. The mentioned adsorbent was used to extract MOF pesticide from aqueous and juice samples by the PT/SPE technique. This study investigated the factors affecting the removal efficiency including elution solvent type, sample volume, solution pH, adsorbent dose, elution solvent volume to remove disturbing factors, and its type and ionic strength.

EXPERIMENTAL
Materials
Aniline monomer (ANI), ammonium persulfate (APS), HCl, and MMT 10K were obtained from Fluca and Merck companies. The MOF insecticide belongs to Dow AgroSciences (Spain). All the solvents used here, such as methanol, water, and acetonitrile, were obtained from Lube Chem.

Instrumentation
In the present project, data were analyzed using a high-performance liquid chromatography (HPLC) system (Smart line model, Knauer Company, Germany) equipped with a Knauer detector (S-2600UV, Germany), a 1000 pump, Knauer degasser, a dynamic mixing chamber, and a C18 column (250 × 4.6 mm). The mobile phase consisted of a methanol/acetonitrile ratio (70:30). The solvent flow rate was 1 ml/min and the volume of each injection was 20 µl. A UV detector was used with a wavelength of 240 nm. CromGate software was employed to record chromatograms and process data. The FT-IR of materials in the range of 4000-400 cm^{1-} was recorded with a Bruker Tensor (model 27, Germany). The morphology of the materials was recorded using a scanning electron microscope (LEO 1430 VP).

EXPERIMENTATION
Synthesis of PANI-MMT nanocomposite
The PANI-MMT nanocomposite was synthesized according to the method reported by Narayanan et al. [10]. To prepare this nanocomposite, the PANI monomer was first sent into MMT and then ANI was polymerized in the matrix. About 1 g of MMT (10K) was first added to 50 ml of water. The resulting suspension was stirred on a magnetic stirrer for 5 h. After 24 h, the suspension was added to a 1 M aqueous HCl solution and stirred for 60 min. The PANI monomer was then added to the suspension on the magnetic
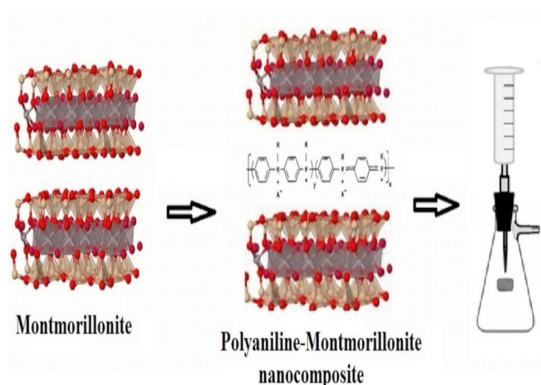
Fig 1: The schematic presentation of PANI/MMT synthesis in extraction device.

Fig 2: SEM image of polyaniline-K10

stirrer. The PANI/MMT ratio was 50 mmol (4.65 g) to 1 g. Subsequently, APS, being dissolved in HCl, was slowly added to the above suspension. The PANI/ammonium ratio was 1 to 1.25. After the reaction completion, the synthesized precipitate was filtered and dried at room temperature after washing. The schematic presentation of PANI/MMT synthesis and application in extraction device is shown in Fig. 1.

Preparation of standard solutions

To prepare a 1000 ppm (1000 mg/L) stock solution, a known amount of the MOF insecticide was dissolved in methanol and then diluted with additional methanol in a flask. Using the prepared stock solution, standard solutions at concentrations of 0.001, 0.01, 0.1, 1, 10, and 100 mg/ml were prepared in methanol. According to the analysis steps, working solutions with a concentration of 10 mg/L were prepared from the standard solutions by diluting with double-distilled water for experiments and were analyzed immediately after preparation. A phosphate buffer solution was prepared at pH 2-10 at a concentration of 0.02 M to adjust the pH. Methanol/water (1: 9), acetonitrile/water (1:9), 0.05% methanol/ammonia solution, and 2 0% methanol/acetic acid were prepared as the elution solvents. Finally, all the prepared solutions, including the stock solution, standards, working solutions, and buffers, were refrigerated in glass vessels at 4 °C.

Preparation of ND/PAN-K10 SPE cartridge

In this method, steel needles were used as a cartridge. First, some cotton was put at the cartridge end to prevent the waste of the adsorbent. Then, 2
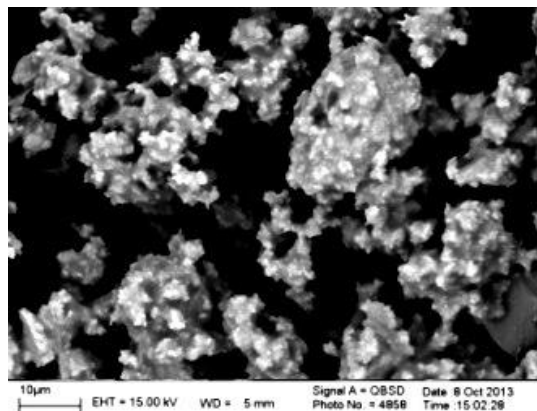
mg of the PANI/MMT nanocomposite was poured on the cotton and shaken vigorously to disperse the adsorbent evenly on the cotton; some cotton was put on it again. To prepare the prepared cartridge for the next tests, the above needle was connected to a syringe, which was then connected to a vacuum system and washed with 1 mL of methanol followed by 1 mL of water. It is noteworthy that the flow rate must be completely controlled to gently and thoroughly wash the adsorbent; at the same time, the cotton is also washed to wash away the disturbing and oily factors by methanol/water.

Extraction process by ND/PAN/K10 SPE

First, the cartridge was prepared with 1 ml of methanol/water. Then, 10 mL of the sample was poured into the syringe and allowed to pass slowly through the absorbent by the vacuum. After the complete passage of the sample through the absorbent and consuming all the contents of the syringe, 1 mL of the 1:9 methanol/water solution was poured into the syringe to remove the disturbing factors in the sample matrix that were adsorbed on the cotton and adsorbent. Afterward, the sample container was placed in a vacuum system under the needle. Then, 1 mL of methanol was poured into a syringe to wash the analyte adsorbed on the adsorbent with the above solvent.

RESULTS AND DISCUSSION

Identification of synthesized samples

The synthesized sample was identified by FT-IR, SEM, and UV-Vis techniques. SEM images were recorded for PANI/K10 nanocomposites, which correspond to the literature [11]. As shown in Fig. 2, the nanocomposite has a homogenous structure
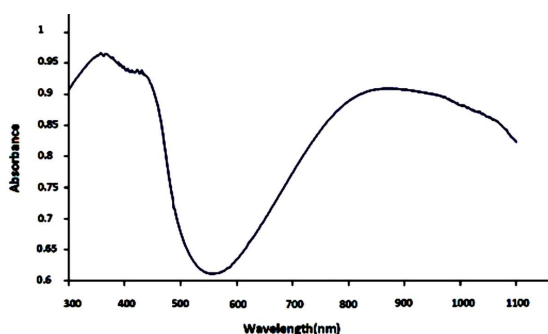
Fig 3: UV-Vis absorption spectrum of polyaniline-K10 nano-composites

Fig 4: FT-IR spectrum related to poly-aniline-K10 nanocomposite

which significantly increases the existing surface area for adsorption.

Fig. 3 depicts the UV-Vis absorption spectrum of a water-dispersed 10K-PANI nanocomposite. The absorption spectrum indicates three peaks at 360, 430, and 800 nm, which belong to the π-π benzoid ring transfer, the doping level, and the polaron band, respectively, indicating that PANI is in a doped state.

Fig. 4 shows the FT-IR spectrum of the 10K-PANI nanocomposite, indicating the characteristic vibrations of both MMT and the polymer. Peaks of the PANI benzoid ring are observed in 1487, 1134, and 801 cm^{-1}. The peaks observed in 1567 and 1215 cm^{-1} belong to the quinoid ring. The Si−O−Si stretching frequency of the MMT matrix, appearing at 1060 cm^{-1}, merges with the nanocomposite peak and appears as a wide peak, suggesting a strong interaction between MMT and PANI in the nanocomposite. The peak of 870 cm^{-1} is caused by the tetrahedral Al (IV) stretching vibration that appears in the nanocomposite. The peak at 1300 cm^{-1} results from the C−N stretching vibration associated with the oxidized (protonated) state of PANI. Therefore, MMT and PANI layers are evidently present, and the slight displacement in the reported values is due to the formation of the composite and the polymer/MMT interactions.

Optimization of the ND/PAN-K10 SPE method

To evaluate the ability of the ND/PAN-K10 SPE method to extract the MOF insecticides, aqueous samples and fruit juices were used as experimental solutions. One of the advantages of this method is the two-way liquid flow in which the liquid solution or elution solution can
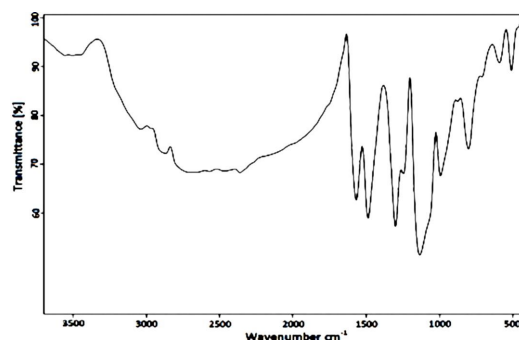
be sucked in or can be pulled out from inside by a syringe piston. Other advantages include less contamination compared to conventional reusable cartridges due to the selective use, very simple preparation, requiring much less time, and cost-effectiveness. More importantly, much less solvent levels are used in this method than the conventional solid-phase extraction. Before optimizing the parameters affecting the extraction, the inhibition time of MOF (the time of MOF peak appearance on the chromatogram) and the suitable mobile phase composition were examined to observe the peak at the appropriate time. To this end, about 30 μl of the stock solution (with the mobile phase composition reported in the literature) was first injected into the device to investigate the peak site, followed by selecting the methanol/acetonitrile mobile phase composition (70:30). A wavelength of 240 nm was also selected according to reviewed articles. After these steps, a calibration curve was prepared by injecting the standard solutions into the device. The parameters affecting the extraction efficiency (elution solvent type, sample volume, solution pH, adsorbent dose, elution solvent volume to eliminate disturbing factors, and its ionic strength) were optimized at the end.

Effect of absorption

Investigating the effect of absorption on the extraction efficiency is considered as a key parameter. Therefore, the results for the effects of different adsorbent doses (0.3-5.0 mg) are presented in Fig. 5, indicating the highest recovery rate using 2.5 mg of the adsorbent. Further increase in the adsorbent dose does not improve the analyte recovery.
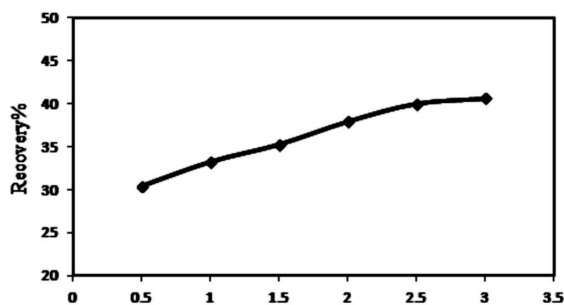
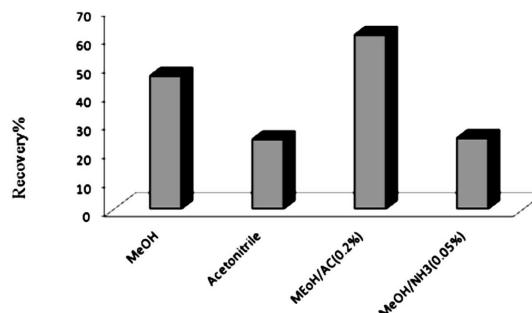Fig 5: Examining the amount of adsorbent on the extraction efficiency

Fig 6: Investigating the effect of washing solvent for the removal of methoxyfenozide from the adsorbent
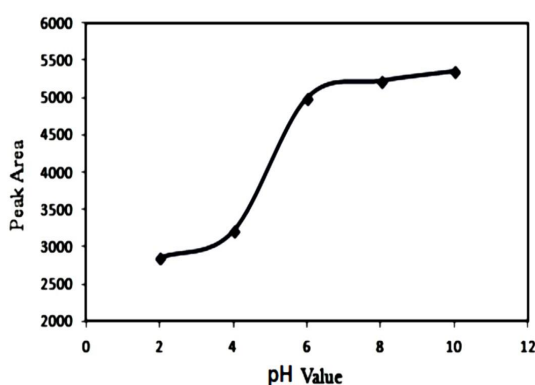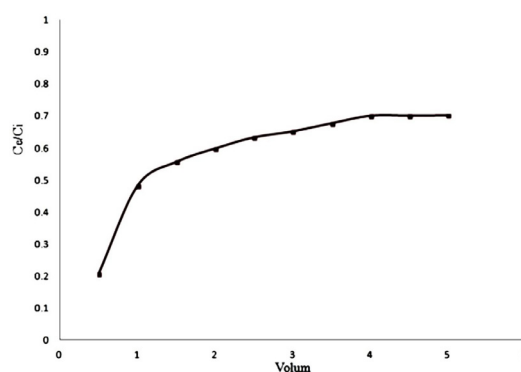

Fig 7: Effect of pH changes on extraction rate

Fig 8: Corresponding curve to BTV

Effect of the elution solvent

An important factor affecting the extraction efficiency is the elution solvent type used to remove or desorb the analyte from the adsorbent. The higher the interaction between the analyte and the elution solvent, the greater the analyte extraction efficiency. Four solvents were tested for the removal of MFO: methanol, acetonitrile, methanol/acetic acid 0.2% (v/v), and methanol/ammonia 0.05% (v/v); the results are shown in Fig. 6, according to which the highest recovery rate is obtained using 0.2% methanol/acetic acid (v/v) as the solvent for analyte removal from the adsorbent.

Effect of sample pH

Since the sample solution pH is an important factor in the transfer of analyte from the sample solution to the stationary phase, the effect of pH on the extraction rate was investigated separately using phosphate from acidic to alkaline values (2-10) . Fig. 7 shows the effect of pH changes on the extraction rate. As shown, a pH value of 6 is the optimal extraction point. Examining the results reveals that the extraction rate decreases at highly

acidic pHs. However, the extraction rate is higher at neutral pHs, and basic or higher pHs have little effects on the extraction efficiency.

Sample solution volume

The breakthrough volume (BTV) curve is displayed in Fig. 8. BTV is defined as the volume of the sample passed through the cartridge until the output value of the cartridge is between 0.01 and 0.05; in other words, 95-99% of the sample remains on the cartridge or the adsorbent.

Elution solvent volume and type

The elution solvent is used to eliminate disturbing factors remaining on the adsorbent; in this condition, the solvent should have no or minimal interaction with the target analyte. In some cases, it can be used for the analyte elution such that it will not interact with disturbing factors and only elute the analyte with itself. In the present project, however, the elution solvent was used for the elimination of disturbing factors to achieve a selective extraction and to reduce non-specific interactions. This was achieved using four types
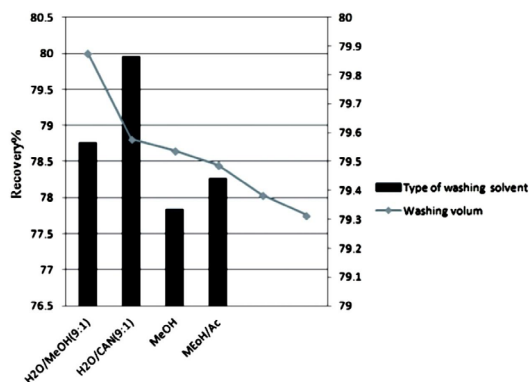
Fig 9: Type and volume of washing solvent used

Fig 10: Effects of ionic strength on extraction efficiency

of solvents: methanol, methanol/water (1: 9), acetonitrile/water (1: 9), and methanol/acetic acid 0.2% (v/v). As shown in Fig. 9, the best results were obtained using the acetonitrile/water solvent. Furthermore, as Fig. 9 shows, the elution solvent volume was examined from 0.3 to 0.5 ml, and a volume of 1 ml was finally selected as the best required volume.

Effect of ionic strength

Ionic strength is often one of the critical parameters in enrichment methods, which was investigated by adding salt to the sample solution. In most conventional methods, such as LLE, SPME, and LPME, increasing the amount of salt improves the extraction efficiency. Generally, an increase in the amount of salt increases the ionic strength of the solution, which in turn probably reduces the analyte solubility in water and, thus, shifts the equilibrium of the reaction toward the adsorbent or organic phase. However, the possible effects of salt addition depend on the physicochemical properties of the analyte and the extraction phase. To investigate the effect of ionic strength, different concentrations of NaCl (0-10% w/v) were prepared in the water sample by maintaining other conditions constant, and the experimental data are presented in Fig. 10.

As shown in the diagram above, the analyte extraction efficiency has risen with increasing the salt concentration. According to these results, it can be explained that adding the salt leads to an increase in the ionic strength of the sample solution, which led to decreased analyte solubility and shifting the reaction equilibrium toward the adsorbent. Thus, the analyte is more adsorbed toward the adsorbent, thereby increasing the adsorption efficiency.
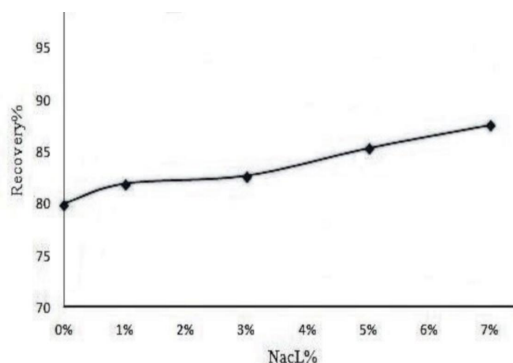
Therefore, a salt concentration of 5% was selected according to the results of data to achieve a higher adsorption capacity in the present project.

Quantitative evaluations and analysis of real samples

Under optimal conditions, the ND/PAN-K10 SPE method was evaluated by testing the degradation parameters of the MOF insecticide in the sample, the results of which are represented in Table 1. For this insecticide, a detection limit of 0.0012 mg/L was calculated based on a signal-to-noise ratio of 3 [12-14]. Using this method, a good linear range was obtained with a correlation coefficient of 0.999. The accuracy of the method was determined by repeating the extraction 4 times from aqueous solutions with a concentration of 10 mg/L under optimal conditions. The relative standard deviation (RSD) was 3.78% using ND/BM/SPNE, suggesting the good reproducibility of this method.

CONCLUSION

The present project is the first report of a needle-based SPE for the quantitative analysis of the ND/PAN-K10 SPE insecticide using a highly sensitive, economical, small, and laboratory-scale method. ND/PAN-K10 SPE coupled with HPLC equipped with a UV detector was developed for the rapid removal of the MOF insecticide from natural water samples. The applied PANI K10 nanocomposite as an adsorbent demonstrates high extraction power. It is also noteworthy that the applied method has a good linear range, reproducibility, and high sensitivity.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

Table1: Comparison of the proposed method with other methoxyfenozide removal methods (QuEChERS method [12], liquid-liquid extraction/liquid chromatography-MS/MS [13], liquid-dispersive liquid microextraction-high-performance liquid chromatography [14].

liquid microextraction-dispersive liquid Ref: 14		Liquid-liquid extraction: Ref:13		QuEChERS/LC-MS Ref:12		Present work				
RSD%	LOD	RSD%	LOD	RSD%	Dynamic Linear Range	RSD%	LOD (µg/ml)	R^2	DLR	Compound
4.1	0.0025	-	0.005	<20	0.005	3.784	0.0012	0.999	0.01-100	Methoxyfenozide

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