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CURRENT TRENDS IN PESTICIDE DETECTION VIA SURFACE ENHANCED RAMAN SPECTROSCOPY (SERS)

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ABSTRACT. As the world population increases, the demand for cheap and adequate food inflates, pushing the farming industry to make use of pesticides in growing amounts year by year. The excess use of pesticides threatens human and wild-life health by direct consumption or in indirect manners via accumulations in soil and ground water. Thus, to monitor the usage of pesticides in farming industry is of utmost importance for the sake of human health preservation of wild-life. Various analytical methods are being utilized in order to detect hazardous substances , however these methods require complex sample preparation procedures, expensive instruments and trained staff. Morever, these instruments are quite disadvantageous since they do not enable on-site analyses because of their massive structures. On the other hand, Surface Enhanced Raman Spectroscopy (SERS), which utilizes metallic nanoparticles enable detection at ultra-low concentrations and it enables on-site analyses with quite simple sample preparation procedures and portable spectrometers. In this review, basics of Raman and SERS is presented, current SERS substrates are explained, basic groups of pesticides are introduced and finally some of the current detection studies conducted via SERS are exhibited.

Keywords: *Pesticide detection, raman spectroscopy, SERS*

YÜZEY GELİŞTİRİLMİŞ RAMAN SPEKTROSKOPİSİ (SERS) YOLUYLA PESTİSİT TESPİTİNDEKİ GÜNCEL TRENDLER

ÖZET. Dünya nüfusu arttıkça ucuz ve yeterli gıdaya olan talep artmakta ve tarım sektörü her geçen yıl artan miktarlarda pestisit kullanmaya zorlanmaktadır. Pestisitlerin aşırı kullanımı, doğrudan tüketim yoluyla veya toprak ve yeraltı sularında birikmeler yoluyla insan ve yaban hayatı sağlığını tehdit etmektedir. Bu nedenle, tarım sektöründe pestisit kullanımının izlenmesi, insan sağlığı ve yaban hayatının korunması adına son derece önemlidir. Tehlikeli maddeleri tespit etmek için çeşitli analitik yöntemler kullanılmaktadır, ancak bu yöntemler karmaşık numune hazırlama prosedürleri, pahalı cihazlar ve eğitimli personel gerektirmektedir. Dahası, bu cihazlar büyük yapıları nedeniyle yerinde analizlere izin vermediğinden oldukça dezavantajlıdır. Diğer taraftan, metalik nanopartiküller kullanan Yüzey Zenginleştirilmiş Raman Spektroskopisi (SERS), çok düşük konsantrasyonlarda tespite olanak sağlamakta ve oldukça basit numune hazırlama prosedürleri ve taşınabilir spektrometreler ile yerinde analizleri mümkün kılmaktadır. Bu derlemede Raman ve SERS'in temelleri sunulmakta, güncel SERS substratları açıklanmakta, temel pestisit grupları tanıtılmakta ve son olarak SERS ile gerçekleştirilen güncel tespit çalışmalarından bazıları sergilenmektedir.

Anahtar Kelimeler: *Pestisit deteksiyonu, raman spektroskopi, SERS*

INTRODUCTION

The growing world population is requiring a rapid and steady increase in food supply, which in return forces the agriculture sector to use various chemicals in order to increase the yield of the agricultural products [1]. Regarding this context, worldwide pesticide consumption nearly doubled between years 1992-2022, from annually 1.8 metric tons to 3.69 metric tons as depicted in Figure 1 [2]. On the other hand, monitoring the use of these pesticides is of great importance since their accumulation in nature causes very critical health issues for human beings, wildlife and the nature itself as well [3].

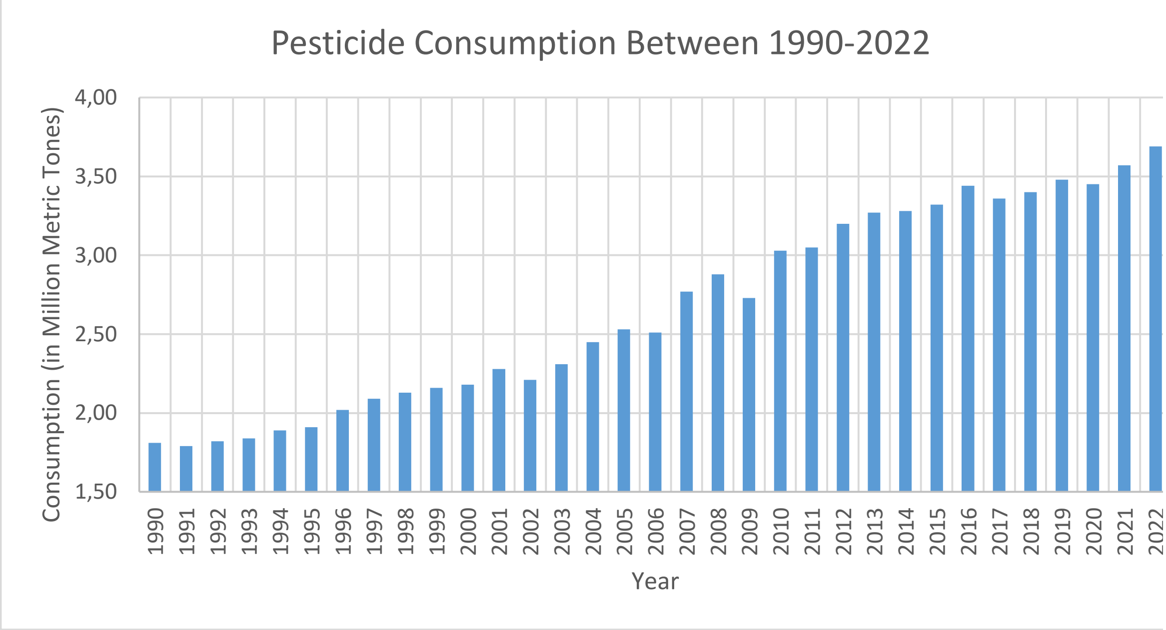

Fig. 1. *Pesticide consumption between 1990-2022*

Thus, detection of pesticide residues with low concentrations is a crucial action in order to ensure public health in regarding food safety issues [4]. Regarding this issue, scientific papers dealing with food safety is displaying a tremendous increase in numbers. The number of papers about food safety has increased from 3200 to 25000 between years 2000 – 2024, as depicted on Graph 2 [5]. Currently, most common techniques applied in such circumstances are High Performance Liquid Chromatography (HPLC) [6-9], Thin Layer Chromatography (TLC) [10-13], Gas Chromatography (GC) coupled with Mass Spectrometry (MS) [14-17], Liquid Chromatography coupled with Mass Spectrometry (MS) [18-21] and ELISA [22-25] These methods, while providing quite accurate results, have various disadvantages such as requiring detailed and time consuming sample preparation procedures, laboratory environment with quite expensive instruments and well-trained analyst staff [26,27].

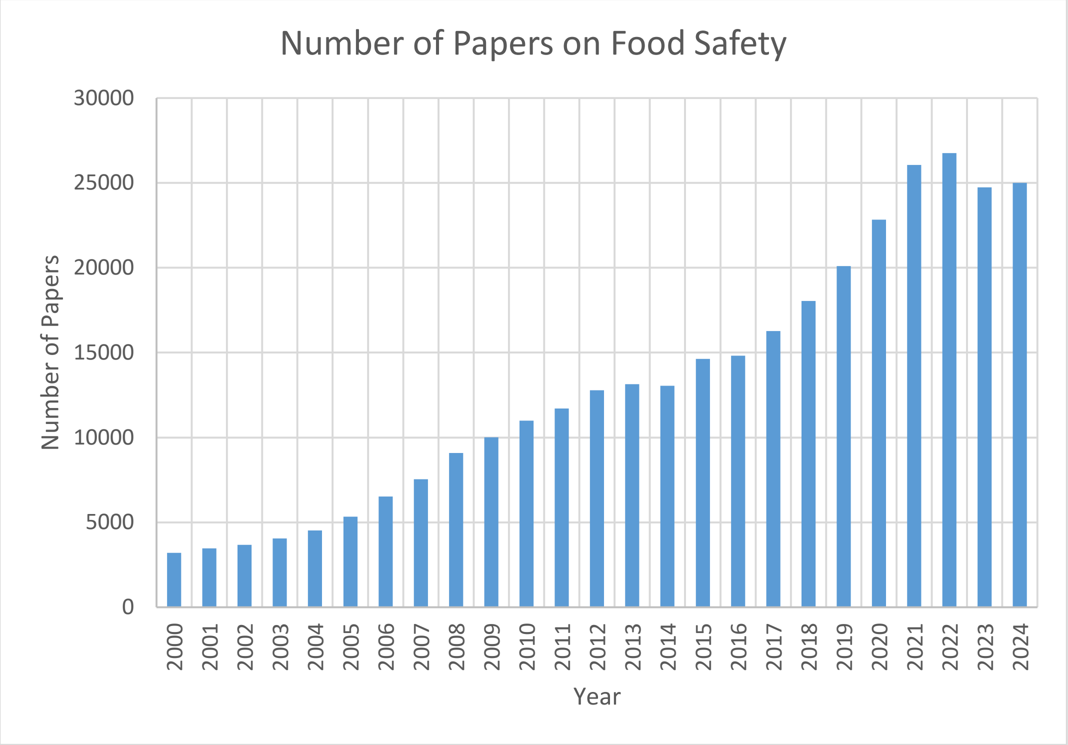

Fig. 2. *Number of papers on food safety between years 2000-2024.*

At this point, Surface Enhanced Raman Spectroscopy (SERS) rises as a promising alternative since it's a non-destructive spectroscopy method which provides fingerprint information about the molecular bonds under investigation [28].

This review paper will firstly discuss the basics of Raman and Surface Enhanced Raman Spectroscopy, then will focus on the current trends on SERS based pesticide detection methods.

Basics of Raman Spectroscopy

The foundation of Raman Spectroscopy is laid with the paper published by C.V. Raman and his colleauge K.A. Krishnan in 1928 [29]. In their revolutionary paper, they'were announcing that they've realized an unfamiliar type of radiation through their scattering set-up. In their configuration, sixty different liquid samples were illuminated with sun light via a telescope with 18 cm. of aperture. After analysing the resulting spectrums, they've realized that a small fraction of the light scattered by the liquids were arriving the detecting screen with a "shift" in their wavelengths, which is known as "Raman Shift" today. Fundamentally, these are the photons which are scattered "inelastically" by the liquid molecules. These "inelastically" photons provide quantitative information about the molecular bonds of the liquid under investigation. If the molecule is left in the "excited state" after the scattering, this is called Stokes Scattering. A very little proportion of the molecules may be in the excited state prior to the scattering process, this time, the molecule is excited to a virtual state, then it may returns to the ground state. This scattering is called "Anti-Stokes Scattering". At room conditions, molecules most probably rest in ground states. Thus, probability of an Anti-Stokes Scattering is fairly low, when compared to Stokes-Scattering.

A third kind of scattering is Rayleigh Scattering, which is basically photons undergoing elastic scatterings with the molecule. In this case, there is no change in the photon's wavelength, thus, this kind of scattering gives no information about the molecular structure [30]. Figure 3 and 4 give the representations of these processes.

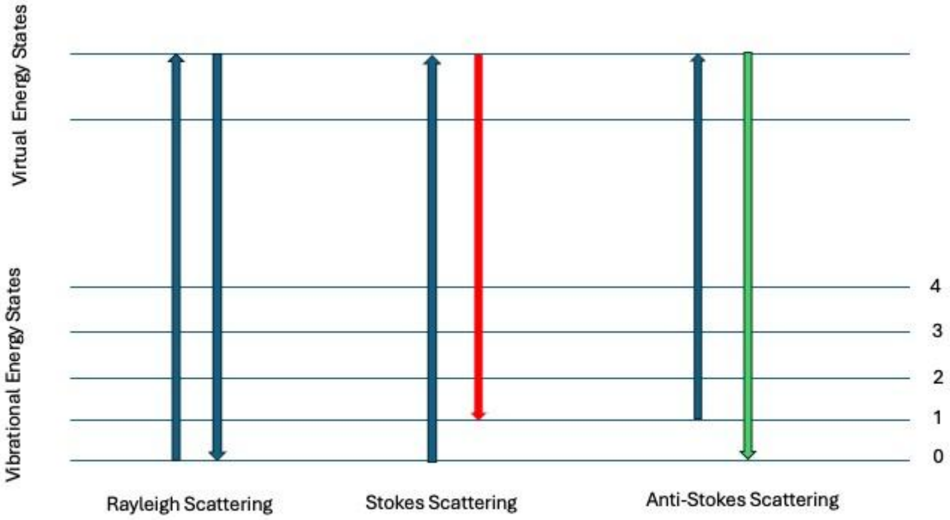

Fig. 3. *Fundamental band transitions*.

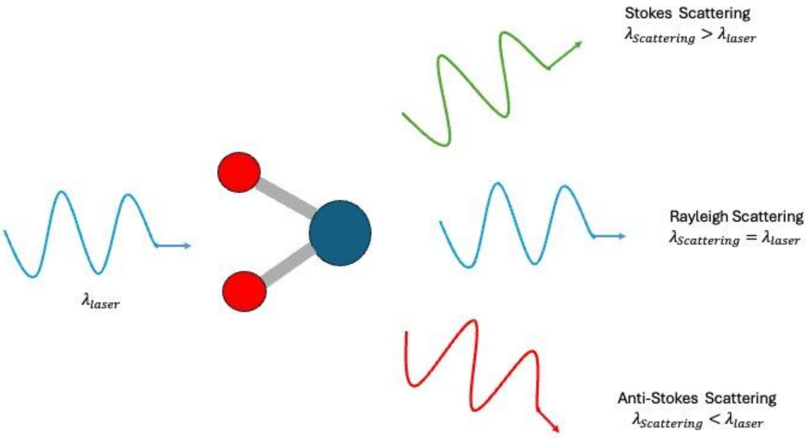

Fig. 4. *Scattering of light through the molecule.*

Fundamentally, here, the shift in the light source's wavelength (or frequency), gives the basic information about the molecular bonds. Here, one can safely conclude that, Raman spectroscopy mainly relies on Stokes Scattering phenomenon, since molecules are mostly in their ground states in room temperature.

The initial setups arranged for Raman Spectroscopy were providing fairly low data since only one in 10^6 scatterings is Stokes, making Raman Spectroscopy disadvantageous [31]. Additionally, they were suffering poor data caused by the poor performance of inferior light sources and optical instruments. However, today, with the striking improvements of laser sources, optical instruments, CCD sensors and electronics, and in addition modern methods such as SERS, Raman spectroscopy provides quite satisfying results in Molecular Spectrosopy [32].

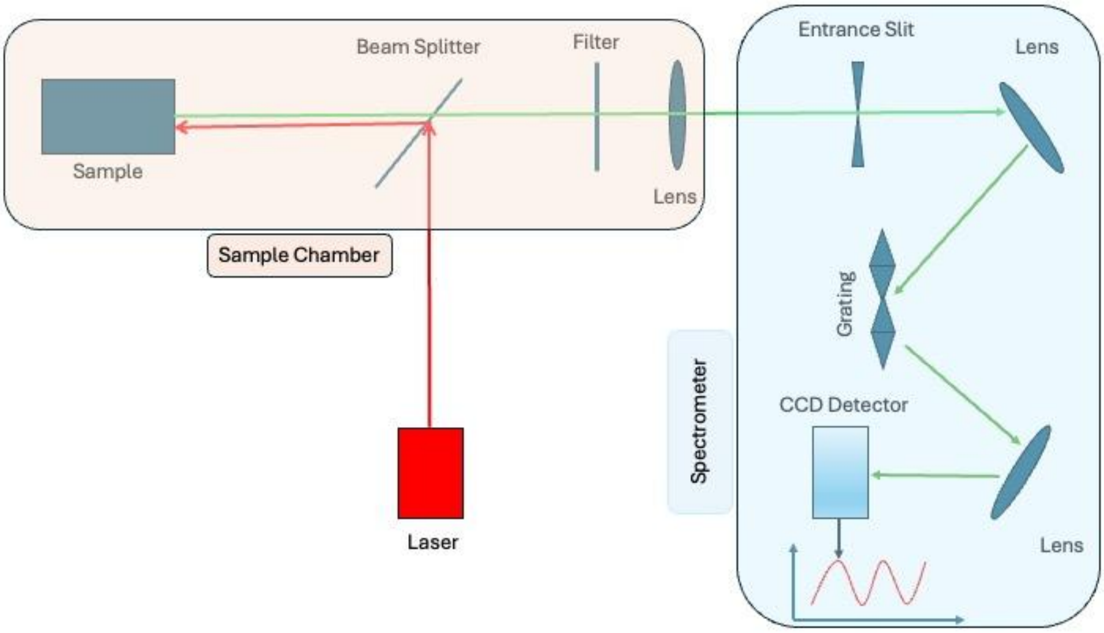

***Fig. 5.** A modern raman spectrometer set-up.*

Figure 5. displays a modern set-up of a Raman Spectroscopy instrument. Basically, one can visualise the system as being composed of two main parts: a sample chamber, and a spectrometer with components such as grating, mirror, lens and ccd detector. Photons leaving a laser source arrive on the sample and following this, scattered photons are directed to the spectrometer through filters and collecting lenses. The grating instruments separates the incoming photons in terms of their wavelenghts/frequencies and transfer the data to CCD sensor and finally electronics reveal the molecular structure under investigation. Today, most common laser sources preferred depending on the research area are 236/532/785 nm for art and archaeology applications, 532/785/1064 nm for biological applications, 514/532/785/1064 nm for forensics applications, and 514/532/785 nm for materials science applications [33].

Basics of Surface Enhanced Raman Spectroscopy (SERS)

Until the study conducted by Fleischmann et. al., Raman spectroscopy had the problems caused by low intensity signals, especially when dealing with samples at low concentrations. In 1974, while they were studying Pyridine molecule on silver electrodes, Fleischmann and his colleagues recognised a noticeable enhancement in the Raman signal intensities [34]. Without making any theoretical clarifications, Fleischmann and his team just commented that the case was related to a kind of enhancement on the analyte surface. The theoretical background of this phenomen, which was named as "Surface Enhanced Raman Spectroscopy" (SERS) later, was revealed by Jean Marie and Van Duyne [35] and Albrecht and Creighton [36] indepedently in 1977. The mechanism simply lays on two separate components, namely, electromagnetic and chemical enhancements, while the electromagnetic enhancement is the dominant one [37]. The electromagnetic component is a result of the electromagnetic fields localized on the substrate material, which is known as “Localized Surface Plasmon Resonance” (LSPR), while the chemical enhancement is simply a result of the change in the polarizability of the analyte molecule [38]. The total enhancement of the Raman signal is the product of these two components, which may reach up to 10^{10} - 10^{11} making detections at even ultra-low concentrations possible [39]. Figure 6 displays the basic mechanisms of SERS, where metallic nanoparticles dispersed around the analyte molecules enhance the raman signal by creating local “hotspots”, in other words, “Localised Surface Plasmons”.

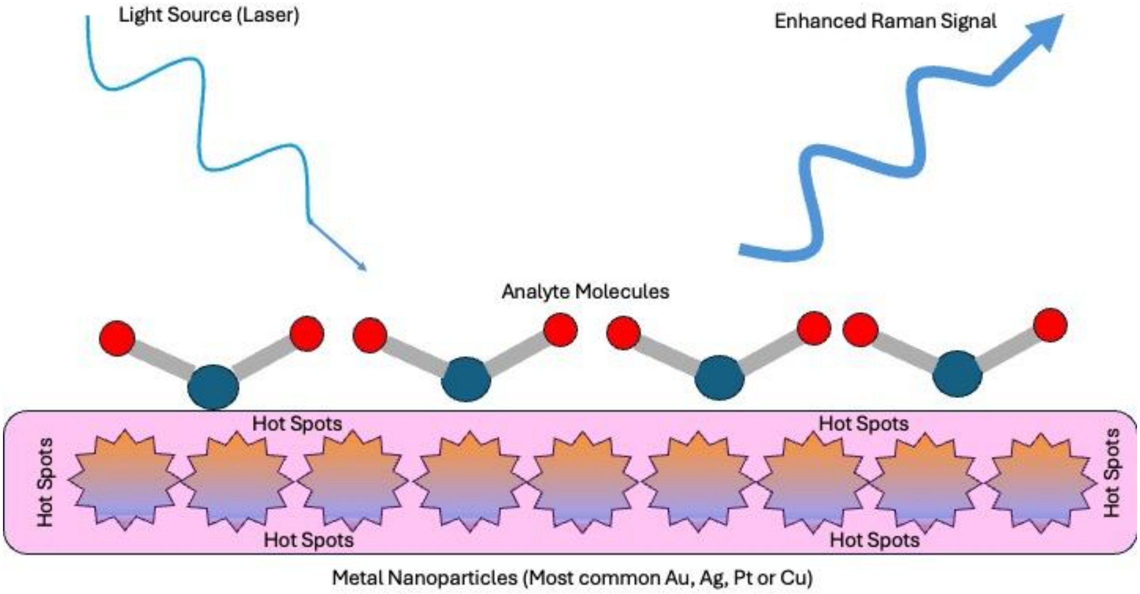

Fig. 6. *Basic mechanism of SERS*

Today, substrate preference and its fabrication is one of the most prominent issues while performing SERS method. In general, there are four common methods followed while seeking the most useful SERS substrate for the analyte molecule under investigation [40].

Nanocolloids

With the aid of reduction agents such as trisodium citrate, spherical silver and gold nanoparticles are manufactured. The most common protocol followed during this process is of Lee and Meisel’s [41] and Turkevich’s [42] method. This method is quite straightforward since the manufacturing process is fairly simple and it’s practical to apply. On the other hand, one can not attain desired reproducibility features when working with these colloids. Another disadvantage of the method is, it is not possible to obtain a homogenenous distribution of ag or au nanoparticles over the analyte moleule.

Roughened Electrodes

In this method, metallic elctrodes are roughened by means of electrochemical methods. Repetitive oxidation and reduction cycles roughen the surface of the metallic electrode which gives a 10^5 – 10^6 enhancement factor [43].

Superficial NP Assemblies

In this method, nanoparticles are deposited on to glass/paper/quartz or silicon surfaces via various methods such as ink-jet printing, screen-printing or dip coating [44-46]. These methods are quite favorable in SERS practices since fabrication process is cheap, the manufactured SERS substrate area is more than sufficient and it is easy to scale the end product.

NP Arrays

This method involves simply depositing metallic nanoparticle layers on a specific surface via lithographic processes [45-48]. In this process, the enhancement factor varies between 10^4-10^7. This process is fairly advantageous in terms of mass production and it provides a better control on homogeneity and reproducibility since it is easier to control the morphology of the substrate [49].

Current Trends In Pesticide Detection via Surface Enhanced Raman Spectroscopy (SERS)

In farming industry, depending on their target organisms pesticides are generally classified into three groups, namely, herbicides, fungicides and insecticides [50]. However, when it comes to detection methods, it is more convenient to re-group them in terms of their chemical structure. Depending on their chemical composition, they are divided into four groups, namely organophosphorus, organochlorines, pyrethroids, and carbamates.

Organophosphorus pesticides are extensively used in farming industry and they are the most risky group for human health. The characteristic oxo-phospate or thiol phospate functional groups are common identifiers fort his class of pesticides [51]. Organochlorine pesticides are basically chlorinated hydrocarbons and pose a great risk to human health by accumlating in soil and ground water[52]. Pyrethroids are most commonly synthesized by duplicating pyrethrin. And are among the least hazardous ones [53.] Carbamates structurally resemble organophosphorus pesticides but they are less hazardous, that’s why they are used more extensively and in gerater amount when compared to organophosphorus pesticides [54].

Below is a Table 1 depicting the most recent trends in pesticide detection literature with the analyte (pesticide) type, preferred substrate and finally limit of detection (LOD) values obtained in the study. Pesticide types are chosen from all four groups in a well-balanced manner, pursuing to seek the most popular ones in the farming industry.

Pesticide	Substrate Utilised	Limit of Detection (LOD)	Reference No.
Thiram	Self Assembled AgNPs	0.024 ppm	[55]
Thiram	Self Assembled AgNP arrays	1.2 ppb	[56]
Thiram	$Fe_3O_4@SiO_2@Ag$ magnetic-plasmonic nanospindles	0.024 ppm	[57]
Thiram	3D spongy flexible nanosheet array	0.2 ($ng \cdot cm^{-2}$)	[58]
Deltamethrin	Ag@ZnO	0.16 µg/kg	[59]
Deltamethrin	Au/Cu2O/ZnO SERS Substrates with Multiple Heterojunctions	0.01 mg/L	[60]
Deltamethrin	Multi-Walled Carbon Nanotubes	0.484 mg/L	[61]
Deltamethrin	AuNPs	0.1 mg/L	[62]
Cypermethrin	Citrate-capped Gold Nanoparticle	0.04 ppm	[63]
Cypermethrin	Nanorough Ag nanoislands/moth wing scale arrays	$1x10^{-10}$ M	[64]
Cypermethrin	Flexible Gold Nanoparticles@Bacterial Nanocellulose	$1x10^{-7}$ M	[65]
Cypermethrin	Hybrid System with Au-Ag Mantis Wings	10 mg/mL	[66]
Malathion	Citrate-reduced Ag NPs	3 ppb	[67]
Malathion	Cellulose Fiber/Structured Plasmonic Silver Nanoparticles	$1x10^{-9}$ M	[68]
Malathion	Ag Nanoparticle Modified PDMS	3.62 µg/L	[69]
Malathion	TbMOF@Au Structure	8.1×10^{-8} M	[70]
Aldrin	Alkyl Dithiol-Functionalized Metal Nanoparticles	$1x10^{-8}$ M	[71]
Aldrin	Electrospun TiO2/Ag Hybrid Structure	$1x10^{-11}$ M	[72]
Carbaryl	PA-6/AuNRs@ZIF-8 Films	1×10^{-9} M	[73]
Carbaryl	In-Situ Grown Silver Nanoparticles on Nonwoven Fabrics Based on Mussel-Inspired Polydopamine	1.0×10^{-12} M	[74]

Carbaryl	Standing Gold Nanorod Arrays	391 ppb	[75]
Carbaryl	Ag/AgNPs with a rose petal PDMS	5×10^{-3} mg/kg	[76]
Acetamiprid	Doped N/Ag Carbon Dots	0.006 µg/L	[77]
Acetamiprid	Fe_3O_4@ZIF-8@Ag nanocomposites	$4x10^{-9}$ M	[78]
Atrazine	A difunctional surface molecular imprinted polymethacrylate probe	0.0012 nmol/L	[79]
Atrazine	Molecularly imprinted polymers coupled with gold nanoparticles	0.005 mg/L	[80]
Thiabendazole	Au@Ag core–shell nanorod	0.034 ppm	[81]
Thiabendazole	Ag NPs encapsulated in dialdehyde starch granules	0.051 ppm	[82]
Thiabendazole	Au nanorods	0.06 ppm	[83]

Table 1. *Various studies to detect pesticide residues via surface enhanced raman spectroscopy (SERS) and corresponding limit of detection (LOD) values.*

RESULTS AND CONCLUSION

The growing demand of the increasing population for food forces the farming industry to harvest much more year by year, which brings the excess use of pesticides against fungi, pests and insects. The growing amounts of these pesticides may directly threaten the human health upon daily consumptions, or may indirectly pose threats by aggregating in food and ground water. Thus, detection of these pesticides in low concentrations is of vital importance. While analytical methods provide quite accurate results, the need for expensive instruments, trained staff and the impossibility of on-site analysis, makes analytical methods quite disadvantageous. At this. Point, Surface Enhanced Raman Spctroscopy (SERS) proves to be a sensible alternative since it does not require complex sample preparation procedures, trained staff and it makes on-site analysis possible via portable spectrometers. In this review paper, kinds of various pesticide classes were introduced, the history and advantages of Raman and. Surface Enhanced Raman Spectroscopy were presented and finally some up-to-date detection studies conducted via Surface Enhanced Raman Spectroscopy were listed. As a final word, one can say that, the growing number of SERS studies dealing with detection of hazardous pesticides in trace amounts keep improving Limit of Detection (LOD), which shows that Srface Enhanced Raman Spectroscopy will prove a robust alternative to analytical methods in future as well.

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