CHAPTER 8: BIOPESTICIDE RESIDUES IN SOIL

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Summary

Biopesticides are considered an alternative to synthetic pesticides due to they possess fewer toxic effects. However, these substances are not regulated and there is a little information about their impact on soils. For that reason, different analytical techniques should be used to control their concentration in soils. The application of a suitable extraction technique is an important step to obtain a high recovery of these substances from soils. Among them, QuEChERS (an acronym for Quick, Easy, Cheap, Effective, Rugged and Safe) is the most used and it is considered as an efficient technique for the extraction of biopesticides. Regarding the analytical techniques, liquid chromatography and gas chromatography coupled to mass spectrometry analyzers, such as quadrupole or triple quadrupole, have been commonly used for their determination. In addition, biopesticides in soils have low levels of persistence and as a result derived substances can be generated. Nevertheless, most of these degradation products have not been studied yet.

8. Biopesticide residues in soils

8.1. Introduction

More than 99.7% of pesticides are accumulated in the environment, undergoing various physicochemical catabolism and biodegradable processes which are strongly linked to the composition and activity of the soil microbial community[1]. Microorganisms play an important role in different soil processes, such as the decomposition of organic matter and the nutrient cycle, which are greatly involved in the function of soil ecosystems[2,3]. Therefore, the loss of the soil microbial community structure can lead to significant changes in fertility, which is a prerequisite for plant growth[4]. For this reason, in the last few years, biopesticides derived from plants, animals, microbes and other natural substances have been used for the pest control in crops minimizing the use of chemical pesticides[5].

Biopesticide registration protocols and dossier requirements slightly differ from country to country, especially in Europe and Organization Economic Cooperation Development (OECD) countries. Different data, such as identification and description of the organism/ingredients, biological qualities, bioefficacies in the laboratory/screen house and field, safety/ecotoxicity studies, toxicology and packaging, are necessary to register biopesticides globally[6]. In the European Union (EU), biopesticides are not recognize as a regulatory category, and are registered as plant protection products (PPPs) under EC 1107/2009. According to this Regulation, biopesticides have only be included in PPP when they present a clear benefit for plant production, and they do not pose any negative effects on the health of human or animals or undesirable environment effects.[7] However, there are fewer biopesticide-active substances registered in EU in comparison with the United States, India or Brazil due to the EU-based biopesticide regulations are more complex[8].

For agricultural purposes, biopesticides are usually applied in higher concentrations than synthetic ones, which pushes them away from the idea that they are completely harmless. As a

result, there is a growing interest in determining their mobility and fate in order to assess their potential of becoming environmental toxins[9]. Previous studies have evaluated the biocidal effects of certain biopesticides in the soil by using different detection tests, as polyphasic microbial assays, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), or soil microbial biomass, among others[4,10,11]. These studies have showed that certain biopesticides can exhibit negative effects on the soil bacterial community. Therefore, the detection, as well as the evaluation of the toxicity of biopesticides in soil is a precondition to improve the regulation of biopesticides in the near future[12]. This chapter covers the analysis and presence of biopesticides in soils. The first section includes soil pre-treatment to recover these substances by using conventional extraction techniques as Shoxhlet[13], solid-liquid extraction (SLE) by shaking[14–16], headspace-solid phase microextraction (HS-SPME)[17], ultrasound assisted extractions (UAE)[9] and QuEChERS (Quick, Easy, Cheap, Effective, Rugged & Safe)[18–21], being this last the most employed extraction technique. The second part collects information about the instrumental analysis employed for the detection of these compounds. Most of them are based on gas chromatography (GC) and liquid chromatography (LC) coupled with various detection systems, such as UV, diode array or mass spectrometry (MS). The last section includes information about the fate of biopesticides in soils by different processes, including the adsorption and degradation, as well as their effects on microbial community.

8.2. Soil sample pre-treatment

Sample pre-treatment is the most important step previous the analytical determination because of the low concentration levels of the biopesticides in soils, their different chemical properties, and the complexity of the matrix. A high recovery of these analytes is needed in order to improve the sensitivity of the method and the reliability of the results[22]. Extraction of biopesticides from soil is based on differences in their physical and chemical properties such

as solubility, polarity, and volatility. In this sense, selective extraction procedures simplify or completely eliminate the sample purification steps required prior to chromatographic analysis[23].

Previous studies have reported the use of different techniques for the extraction of biopesticides from soils (Table 8.1). The conventional Soxhlet extraction has been used to extract azadirachtin A from soil. This method required high solvent consumption (200 mL) and a long extraction time (6 h)[13]. Therefore, other efficient techniques have been employed for the recovery of certain biopesticides in soils as SLE technique by shaking. This has been used for the extraction of rotenone, using 10 mL of ethyl acetate as extraction solvent and an extraction time of 30 min, obtaining high recoveries (95-100%)[14,15]. Feng et al.[16] reported the determination of pyrethrin residues, including pyrethrin I and II, cinerin I and II, and jasmolin I and II in turnips (turnip leaves, turnip tubers, and the whole of plant) and cultivated soil. The extraction was carried out by vortex agitation of soil with 20 mL of acetonitrile and 3 g of NaCl for 2 min. The dry extract was redissolved with 1 mL acetone + *n*-hexane (1:9; *v/v*) for solid phase extraction (SPE) cleanup, using 1 g of anhydrous sodium sulfate (Na_2SO_4), which was added into a florisil cartridge (SPE Cartridge, 500 mg, 3 mL), and suitable recoveries were achieved (88–104%).

A recent study used HS-SPME to recover the volatile metabolomics residues (xenometabolites) that were released after the application of *Myrica gale* methanolic extract in soils[24].This study employed as optimal conditions 5 min for incubation time, 30 min of extraction time, 40°C of extraction temperature and 50/30 µm Divinyl-benzene/Carboxen/Polydimethylsiloxane as fiber coating. The extracted compounds were desorbed and introduced in the analytical instrument.

UAE is another fast and efficient technique for extracting biopesticides. The high yield from UAE is due to the cavitation, which allows the disruption of the cell wall by the ultrasound waves[25]. López-Serna et al.[9] reported the use of UAE during 20 min for the extraction of cinnamaldehyde (CAD) and diallyl disulfide (DAD) in soil by using different solvents, achieving the highest recovery rates of 70 and 61% for CAD and DAD, respectively when ethyl acetate was used.

<Table 8.1 here>

QuEChERS is an extraction and clean-up technique that has shown higher recoveries for different classes of compounds including biopesticides, chloroalkanes, phenols, and perfluoroalkyl substances[22]. For instance, Drozdzyński and Kowalska[18] determined azadirachtin, rotenone, spinosyn A, and spinosyn D for the first time by using a citrate buffered QuEChERS extraction method followed by a clean-up step by dispersive SPE (d-SPE), using primary secondary amine (PSA) and octadecylsilyl (C18). The method employed 5 g of soil, 5 g of water and 0.1% of acetic acid in 10 mL of acetonitrile (MeCN) and the mixture was shaken during 5 min. Recoveries higher than 83% were achieved with this extraction method. Another study reported the determination of 15 biopesticides in soil including nicotine, sabadine, veratridine, rotenone, azadirachtin, cevadine, deguelin, spinosad D, pyrethrins and piperonyl butoxide in agricultural soils[19]. This study evaluated different extraction procedures such as SLE using mechanical shaking, sonication, pressurized liquid extraction (PLE), and modified citrate QuEChERS. Ethyl acetate was used for SLE sonication and PLE, whereas water and 1% of acetic acid in acetonitrile was used for QuEChERS (Table 8.1). Recovery values were 30-110% when QuEChERS was tested, which were higher than those obtained with SLE (3-37%) and PLE (3-53%) for all compounds as it can be observed in Figure 8.1. Therefore, this approach was chosen as the most suitable extraction technique for the determination of biopesticides[19]. El-Saeid et al.[20] applied a non buffered citrate QuEChERS method to extract

spinosad from soils. For that, 10 g of soil with 7 mL of water were shaken for 25–30 min, and then MeCN (10 mL) was added to the samples, and the final mixture was shaken for 5–6 min. A clean-up step based on d-SPE, using C18 as sorbent, was utilized obtaining recoveries from 98 to 102%. Huang et al.[21] extracted and purified carvone from soil using a QuEChERS-based method. Briefly, 5 g of soil was placed into a polypropylene tube with 10 mL of *n*-hexane and 5 mL of ultra-pure water and vortexed for 2 min. Then, the extract was cleaned-up with a polypropylene tube containing 40 mg of C18 and 100 mg of $NaSO_4$ and shaken with vortex for 2 min. This method provided recoveries from 95.7 to 104.2%[21]. d-Limonene has also been extracted with the same mixture of solvents as in the previous study and the extract was purified with C18 and graphitized carbon black (GCB), achieving recoveries from 71.2 to 114.5%[26].

<Figure 8.1 here>

Comparing the extraction techniques, QuEChERS showed better biopesticide recoveries with less extraction solvent (1/1 and 1/2 (*w/v*)) versus other common extraction techniques as Soxhlet, SLE or UAE (1/2-3/5 (*w/v*))[13–15,18,19,27]. It was also observed lower values of RSD for the determination of nicotine, sabadine, veratridine, rotenone, azadirachtin, cevadine, deguelin, spinosad and pyrethrins by QuEChERS in comparison with SLE and PLE[19]. It is important to note that the extraction of pyrethrins by an assisted agitation SLE with vortex employed acetonitrile in the same ratio solid-to-solvent as QuEChERS (1/1 (*w/v*)), obtaining similar recoveries with a shorter extraction time (1-2 min)[16,19]. In addition, previous studies reported similar recoveries of 95-100% for rotenone by assisted agitation SLE with ethyl acetate in the same ratio as QuEChERS (1/2 (*w/v*))[14,15,19]. However, QuEChERS needed a rehydration step of the soil and this can last up to 30 min, increasing the overall extraction time[19]. Other studies added the water with the extraction solvent without a previous hydration step reducing the sample preparation[21,26]. In summary, QuEChERS could be the best option for the biopesticides extraction from soils. For that purpose, an optimization of extraction parameters, such as the

ratio sample-to-solvent, solvent type and the extraction time, is needed to establish the best conditions for the highest recoveries of specific biopesticides from soils. The most employed extraction solvent in QuEChERS was a mixture of 1% of acetic acid in MeCN for the recovery of azadirachtin, rotenone, spinosyn A and D, nicotine, sabadine, veratridine, rotenone, azadirachtin, cevadine, deguelin, spinosad and pyrethrins[18–20], whereas *n*-hexane was recently used to extract d-limonene oxide isomers, (-)-carveol isomers, and (-)-carvone[21,26]. The ratio of solid-to solvent was from 2/1 to 1/2 (*w/v*) and the time of extraction was ranged from 1 to 6 min[18–21,26].

8.3. Instrumental analysis

After a suitable sample preparation, instrumental analytical methods are applied to provide key information about the composition of biopesticides in processed soil samples. GC and LC are the most used methods providing a quantitative analysis with good resolution. Classical detectors provide limited sensitivity and information on the compounds present in the samples. Therefore, MS is commonly coupled to chromatographic techniques, providing a high information to identify compounds based on their mass-to-charge (*m/z*) values. Then, the main analytical methods employed for the detection of certain biopesticides in soils are described.

8.3.1 *Chromatographic methods*

GC has been used for the analysis of biopesticides in soil coupled to MS analysers due to most of biopesticides present a high volatility[13]. However certain biopesticides, such as rotenone, possess a low volatility and/or thermolabile characteristics, so they are analysed by LC. Table 8.2 shows an overview of the main characteristics of the developed GC and LC methods employed for the detection of certain biopesticides in soils. It can be observed that (5%-phenyl)-methylpolysiloxane phase (HB-5MS or HP-5MS GC columns) was the stationary phase commonly used to separate biopesticides, including cinnamaldehyde and diallyl disulfide, limonene oxide isomers, (-)-carveol isomers, carvone, pyrethrins and

xenometabolites, whereas 5% phenyl polysilphenylsiloxane was the stationary phase used to separate spinosad. Columns with different lengths, between 30 and 60 m, can be used, with and internal diameter of 0.25 mm and 0.25 µm of film thickness[9,16,20,21,24,26]. These stationary phases have provided an excellent separation and robustness for analytical applications, delivering excellent inertness with active compounds. In addition, the injection volume ranged from 1 µL to 5 µL, whereas flow rate was usually equal to or lower than 1.5 mL/min, when MS was used, and 15 mL/min when flame ionization detector (FID) was applied for the detection of azadirachtin A[13]. The splitless injection mode allowed the detection of several biopesticides that were found at low quantity in soils improving the sensitivity of the developed method. Nevertheless, split injection, with a ratio 5:1, has showed to be suitable to analyse limonene oxide isomers, carveol isomers, and carvone due to their concentration is high enough to be detected (LOD of 2-16 µg/kg and LOQ 6-50 µg/kg)[21,26].

In the case of LC, reversed phase is applied with C18 as stationary phase, utilizing columns with lengths ranging from 100 to 250 mm[14,18,19,27]. In general, it should be noted that when reversed phase is used, nonpolar compounds as pyrethrins are strongly retained, whereas polar compounds such as nicotine are slightly retained. Conventional high performance liquid chromatography (HPLC) had been used in previous investigations, by using stationary phase with particle sizes of 5 µm, providing an analysis time of more than 15 minutes for the analysis of a single compound (rotenone)[14,15,27]. The use of columns filled with sub-2 µm particles (ultra-high performance liquid chromatography, UHPLC) provided better chromatographic resolution than conventional HPLC, reducing analysis time, obtaining narrower peaks, increasing signal-to-noise ratio and improving the sensitivity of the analytical method. Therefore, these properties enabled simultaneous determination of more than one compound in less than 10 minutes[18,19]. The composition of the mobile phase affected the separation of analytes, influencing their retention times, selectivity between these and the peak efficiency. A

mixture of acetonitrile or methanol and water is commonly used as mobile phase. Some additives can be added to the water, as ammonium acetate[18], ammonium formate[19] or trifluoroacetic acid[15], to maintain a relatively constant pH upon dilution to improve the ionization, avoiding retention and selectivity changes[27]. The isocratic elution mode has been used for the determination of rotenone (Table 8.2), although gradient profile has been commonly employed for the simultaneous determination of several biopesticides to reduce analysis time[18,19,28]. Finally, it is important to note that injection volume ranged between 5 µL to 100 µL, whereas the flow rate was 0.3-0.4 mL/min with the MS detection, whereas it was 1.0-1.5 mL/min with the UV or DAD detection (see Table 8.2).

<Table 8.2 here>

8.3.2 *Detection*

Traditional analytical methods have mainly used UV and DAD detectors, coupled with HPLC, or FID coupled with GC, for the determination of biopesticides in soils. Thus, rotenone was determined by UV detection at 295 or 299 nm[14,27] or using DAD at 295 nm[14]. Other biopesticides, such as pyrethrins, were detected at 230 nm[28]. GC-FID has been used for the determination of azadirachtin A in soils[13]. However, HPLC-UV and GC-FID only provided the retention time at which the compounds eluted from the column, but this information was not enough for the reliable identification of the targeted compounds, since other substances, coextracted from the matrix, could be present and eluting at the same retention time. Taking into account this fact, MS must be used for high sensitivity and unambiguous detection, confirmation, and determination of the analytes.

In consequence, conventional detection has been replaced by MS[18] improving the sensitivity and the selectivity of the developed methods. In GC, the most used mass analyzer is single Quadrupole (Q), equipped with an Electron Impact (EI) ionization system for the detection of biopesticides in soils (Table 8.2). The main benefits of this analyzer are the large dynamic

range, its high scan frequency, and the ability of the EI to obtain reproducible fragmentations for the analysed compounds. A previous research analysed pyretrhins by using this technology by selective ion monitoring (SIM) mode, using Q as analyzer, allowing their identification by monitoring different ions[16]. For instance, cinerin I, jasmolin I and pyrethrin I have a common fragment at *m/z* 123 with the highest abundance, but these compounds are distinguished by other fragments ions which are less abundant, (*m/z* 150 and 168 for cinerin I, *m/z* 69 and 135 for jasmolin I and *m/z* 81, 105 and 162 for pyrethrin I). On the other hand, cinerin II and jasmolin II had the same fragment ions (*m/z* 107, 93, 121, 167), but were distinguished by different retention times due to jasmolin had one more methyl group in the molecule, and therefore eluted later than cinerin[16].

Huang et al.[26] reported a rapid and sensitive GC–MS method for the simultaneous determination of d-limonene and its oxidation products (cis-limonene oxide, trans-limonene oxide, cis-(-)-carveol, trans- (-)-carveol, and (-)-carvone in soils. Chromatograms and mass spectra of d-limonene and its oxidation products with their quantifier ions, confirmatory ions, and retention times are showed in the Figure 8.2. It can be observed that the isomer cis and trans of limonene oxide have the same fragment ions at *m/z* 43, which is the most abundant, whereas the less abundance ones are *m/z* 41 and 67. They can be distinguished by their different retention times, eluting the cis isomer before the trans compound. Nevertheless, cis and trans carveol presented one different fragment with less abundance (*m/z* 55 and 41, respectively) which allowed the distinction among them.

<Figure 8.2 here>

Recently, GC-Q was also applied for characterizing unknown metabolites by fast spectral library search and/or by structural elucidation[24]. This research characterized 96 xenometabolites after the application of a *Myrica gale* methanolic extract in soils to study the dissipation of its volatile residues by using a Q mass analyzer and spectral library for structural

elucidation. Among them, 63 compounds were identified for the first time (47 bioherbicide components and 16 degradation by-products). Six of the most abundant biopesticides were eucalyptol, L-terpinen-4-ol, α-terpineol, α-terpineol acetate, 3,7(11)-selinadiene and germacrone. The rest of the xenometabolites were mainly terpenes, aromatic and aliphatic esters, alcohol and ketones. The fast identification was carried out by using the NIST library for EI-MS fragmentation spectra and the Kovats retention index (RI) calculation. The detection of numerous metabolites was possible by the fragmentation with EI of the parent compounds through this simple spectral library NIST despite the low resolution of the Q analyzer. In addition, Kovats RI calculations ensured higher identification confidence. Therefore, this method has showed to be a useful tool to study the environmental fate of volatile xenometabolites in emerging complex biopesticides.

Moreover, a recent research employed a triple quadrupole analyser (QqQ) for the detection of pesticides, including biopesticides in soils[20]. This method allowed the detection of 14 pesticide residues that belonged to organochlorine pesticides (OCPs), organophosphates (OPPs), pyrethroids, carbamates, and biopesticides. Among them, the spinosad biopesticide was detected in these soils. Therefore, the use of GC with QqQ has showed to be a sensitive and effective method for screening and monitoring biopesticide residues. Finally, it should be noted that GC coupled to Q and QqQ analyzers allowed the quantification of biopesticides from soils with quantification limits (LOQs) between 6 to 82 µg/kg (Table 8.2).

In the case of LC, the ionization of biopesticides is carried out by atmospheric pressure ionization (API), applying either electrospray (ESI)[29,30] or atmospheric pressure chemical ionization (APCI)[31]. APCI has shown better results for certain compounds such as rotenone and pyrethrins[32], observing that it was less affected by the matrix components than ESI. However, a lower sensitivity was obtained from APCI interface compared with ESI. On the other hand, suitable sensitivity was obtained for the analysis of azadirachtins, slannine and

nimbin from neem extracts using ESI[33]. Schaaf et al.[34] reported the determination of azadirachtin by using several ionization conditions, observing that APCI provided better results. Nevertheless, a recent research used ESI for the ionization of azadirachtins from seed and leaf extracts of *Azadirachta indica*[30] and pyrethrins in environmental samples obtaining a high sensitivity[29]. In addition, in the case of analysis of biopesticides in soils, ESI has been the ionization system utilized (Table 8.3), using Q or QqQ as analyzers. For instance, Cavoski et al.[14,15] reported the use of LC coupled with both analyzers to detect rotenone and its main product of photodegradation (12aβ-hydroxyrotenone) in soils. Rotenone presented *m/z* 395 $[M+H]^+$ and 436 $[M+ H^+CH_3CN]^+$ adducts, whereas 12aβ-hydroxyrotenone gave the *m/z* 393 $[M+H-H_2O]^+$ adduct. These were identified and confirmed by LC/MS analysis monitoring in the single-ion mode, the ions 395 and 393 *m/z*. However, Q was not able to distinguish an analyte from other ones or interferences with overlapped retention time (t_R) and the same *m/z*. Consequently, a simultaneous detection has been carried out by QqQ analyzer, which allowed the monitoring of two or more different mass transitions (precursor ion > product ion) in many analytes. Currently, "qualifier ions" in addition to the "quantifier ions" are used as an alternative to mass transitions to exclude interference in individual samples based on a typical constant ratio in the number of ions from both transitions[35]. The multiple reaction monitoring (MRM) mode of the QqQ allowed the selection and detection of target pesticides with an improvement of the selectivity[36]. Drożdżyński et al.[18] reported a method for the simultaneous analysis of three organic farming bioinsecticides (azadirachtin, rotenone, spinosyn A and D) in soil samples by UHPLC-MS/MS. This research performed the selection of specific MRM transitions for each analyte. Therefore, this proposed methodology allowed the selective determination of selected biopesticides residues at trace levels with a great analytical performance. In addition, Prestes et al.[19] employed UHPLC-MS/MS for the simultaneous analysis of more than 10 biopesticides residues in soil samples. It is important to note that the

precursor ion of azadirachtin corresponded to the sodium adduct ion (*m/z* 743) $[M+Na]^{+}$[37]. In the case spinosyn A and B, these had a precursor adduct $[M+H]^{+}$ (*m/z* 732 and 746) and they had the same product ions at *m/z* 142[18]. It was also worth emphasizing that in the case of pyrethrins, the class II had the $[M+H]^{+}$ and $[M+H+CH_3CN]^{+}$ adducts, whereas those that belonged to the class I also provided the adduct $[M+H_2O]^{+}$[38]. Furthermore, each pair of pyrethrins had the same two main product ions (*m/z* 161 and 133 for pyrethrin I and II, *m/z* 149 and 107 for cinerine I and II, and *m/z* 163 and 107 for jasmoline I and II) when $[M+H]^{+}$ was selected as precursor ion[19]. However, certain compounds such as rotenone and deguelin had the same precursor ion (*m/z* 395) and product ion spectra, thus, their determination was carried out considering their different retention times when simultaneous determination was performed[19]. Finally, it should be mentioned than when LC was combined with MS or MS/MS, low LOQs ranging from 1 to 15 µg/kg were obtained (Table 8.3).

<Table 8.3 here>

8.4. Fate and mobility of biopesticides in soils

The fate and mobility of biopesticides in soils imply complex mechanisms that are influenced by a variety of processes including volatilization, leaching, adsorption/desorption and degradation by physical, chemical and biological processes, which are provided when biopesticides are released to the environment[39,40]. Among them, adsorption and degradation are the key processes to predict the fate of biopesticides in soils. The soil adsorption coefficient (K_d) is a measurement of the quantity of chemical substance adsorbed onto soil per amount of water. Because adsorption is mostly accomplished by partition into soil organic matter, K_d is commonly normalized to the soil's organic carbon content and express the distribution coefficient as K_{oc}. This is known as the organic carbon-water partition co-efficient (Equation 8.1).

$$\mathrm{Koc} = (\mathrm{Kd} \times 100)/\%\ \mathrm{Organic\ carbon} \quad \text{(Equation 8.1)}$$

K_{oc} is commonly estimated considering octanol-water partition coefficient (K_{ow}) and water solubility. Generally, it has been found that adsorption of biopesticides is positively correlated with octanol-water partition coefficient and negatively correlated with their water solubility[20,39]. Therefore, K_{oc} will be a measure of the soil adsorption and it is useful to predict the mobility of organic soil compounds. For that reason, larger K_{oc} values indicate that biopesticides are strongly bound to the soil. Table 8.4 presents a summary of K_{oc} as well as the time to 50% disappearance (DT_{50}) of the most important biopesticides.

<Table 8.4 here>

Biopesticides are non-persistent under the field conditions and most of them are mainly degraded by light and temperature[41]. In this sense, transformation products are generated by degrading biopesticides through a series of complex chemical reactions. In this sense, rotenone was widely studied, and its degradation depends on photolysis, soil properties including organic matter and clay concentration and temperature[14,15,27]. A study reported a fast initial degradation of rotenone with DT_{50} varied from 5 to 7 h by photolysis reaction[14]. Another study reported the half-lives of rotenone, which ranged from 1.98 to 2.76 days in soil[27]. Different reactions, such as O-demethylation, epimerization, epoxidation, hydroxylation and dehydration, are involved as a result of the photolysis. In addition, a higher degradation of rotenone is provided when the soils contain a higher organic matter[14]. Moreover, Cavoski et al.[15] revealed that an increase of 10°C in temperature provided a decreasing in the DT_{50} value by a factor of 4.2 for rotenone, being its main degradation product 12 aβ-hydroxyrotenone[14].

In the case of azadirachtins, azadirachtin A has a low to very high mobility (K_{oc}= 20.6–875.1 L/kg), but there is no information on K_{oc} for azadirachtin B although, it is believed that azadirachtin B has similar adsorption/desorption endpoints as azadirachtin A[42]. Their degradation followed first order kinetics with different half-lives, depending on several factors as temperature. The persistence of azadirachtin A and B was determined at two different

temperatures (15 and 25°C) after application of the commercial neem insecticide to soil, observing that temperature affects to the degradation rates. The DT_{50} for azadirachtin A was 19.8-43.9 and 20.8-59.2 days for azadirachtin B at 15ºC and 25ºC, respectively[43]. Moreover, two unknown degradation products were commonly observed in soil although they were not identified by HPLC-UV at λ = 215 nm. Other studies evaluated the effect of azadirachtin in soil, observing that the population of bacteria, actinomycetes and diazotrophs, was not affected because of the addition of azadirachtin on culturable soils[44]. Other studies reported that azaridachtin had no significant negative effects on arbuscular mycorrhiza populations[45]. Additionally, Suciu et al.[46] also evidenced that trifloxystrobin and azadirachtin did not provide adverse effects on soil microbial functions even at high dose rates. Recent studies have revealed that although azadirachtin is considered environmentally safe due to its biological origin, it has adverse effects on rhizospheric bacterial and fungal communities at different plant growth stages, which was similar to synthetic pesticides[11,47]. Furthermore, as demonstrated by their genes and transcripts, azadirachtin has detrimental impacts on plant growth promotion, nitrogen fixing bacterial community, and nitrification. Therefore, the content of azaridachtin in soils should be regulated due to its adverse effects on the soil[11].

Spinosyn A and D are transformed to spinosyn B (metabolite of spinosyn A) and N-demethylated spinosyn D (metabolite of spinosyn D) in soil. DT_{50} values were ranged from 6.5-46.3 days for spinosyn A and 11.3-62.6 days for spinosyn D, which depends on the soil conditions[48]. Furthermore, it was studied that half or more of the spinosad was adsorbed to the interior of soil particles and it is not available for photodegradation[48]. Moreover, spinosyn A and D, and spinosyn B presented a low mobility to no mobility in soils, whereas the N-demethyl spinosyn D metabolite exhibited medium mobility to no mobility in soils. It was concluded that the adsorption of spinosyn A and D and its metabolites was not pH dependent[48]. Moreover, spinosyn A had a value of K_{oc} = 35024 L/kg, whereas K_{oc} value is not available for spinosyn

D although it is assumed that this had the same sorption characteristics as spinosyn A[49]. The dissipation of spinosad in soil was evaluated after the application of a spinosad formulation (Tracer®45.5 % SC) sprayed in the field at two doses of 51.0 and 102.0 g active ingredient (a.i.)/ha in 500 L water at 50 % fruiting stage. The DT_{50} in soil were 6.36 and 6.91 days for the recommended dose and the double one respectively. In addition, a second spray of similar treatment was done after 15 days of first spray obtaining DT_{50} between 5.49 and 6.76 days[50]. The dissipation of spinosad was 98.1 and 76.9 % by 15 days for the recommended and double dose, respectively. A recent study reported the effects of sub-lethal doses of two insecticides, a biologically-derived (spinosad) and a synthetic organophosphate (chlorpyrifos), on earthworm *Eisenia foetida* and microorganisms in organic soil[51]. Early DNA damage was estimated in earthworms exposed to chlorpyrifos, while the impact of spinosad was only significant at the end of the toxicity test.

In relation to sabadilla alkaloids (cevadine and veratridine), their degradation were faster under photolysis (hours) than under hydrolysis , since hydrolysis is expected to occur at much slower rates (days to years)[52]. K_{oc} values ranged from $6.1 \cdot 10^4$ (sabadinine) to $2.3 \cdot 10^6$ L/kg (veratridine). In addition, nicotine is usually degraded to cotinine and presented DT_{50} and K_{oc} values in soils of 0.5 days and 100 L/kg[53] respectively. Bulenga Lisuma et al.[54] indicated that nicotine sorption isotherms fitted a Freundlich model, revealing its adsorption was based on the soil depths (0–50 cm), ranging from 2.81 to 4.61 mg/kg in sandy loam and sandy soils, respectively. The nicotine desorption was ranged from 0.89 to 1.12 mg/kg in loamy sand and sandy loam soils, respectively.

Pyrethrins were strongly adsorbed into soil surfaces and were commonly considered no mobile with a DT_{50} between 1.9 to 97.2 days[55,56]. It was observed that pyrethrins are degraded very quickly upon exposure to sunlight and they did not persist in the environment beyond a few weeks[16].The half-life of pyrethrin I and II was less than two hours in field conditions, whereas

under dark conditions, there was little degradation over time[57]. Moreover, the sorption of pyrethrins into soil increased with raising soil organic matter. Thus, it was found that soils containing two times organic matter content than native absorbed more pyrethrins, and their mobility was reduced by humic acids[28]. Feng et al.[16] revealed that the pyrethrins dissipation was 1.0–1.3 day. This study showed a fast degradation of pyrethrins in greenhouse in comparison with open field.

Regarding the monoterpenoid biopesticide compounds, as α-terpineol, limonene, thymol, menthol, carvone, eucalyptol, and perillaldehyde, they are aromatic and volatile at near-room temperature[21]. Monoterpenoid biopesticides were susceptible to oxidation, cyclization, isomerization, dehydrogenation, and other breakdown reactions, and as a result of these features, they were not environmentally stable[21,58]. For instance, carvone, limonene oxide, carveol, and limonene hydroperoxide were all formed when the limonene monoterpenoid was oxidized in the environment[21]. Carvone had a high mobility in soil of 111 L/kg and it was not persistent with a DT_{50} ranging from 0.2–5 days[59]. Huang et al.[26] reported the production of d-limonene oxidation products under indoor simulated conditions (Figure 8.3.), which was fast dissipated within 7 days after its application in soils, and some oxidation products were generated. The production of *trans*-compound isomers was higher than their *cis* isomers in three soils, Jilin (S1), Jiangxi (S2) and Sichuan (S3). Monoterpenoids were degraded mostly by microbes in soil and water, with a small percentage lost by leaching and evaporation[60]. Environmental factors, such as soil type, pH, temperature, humidity, and precipitation, will influence this degradation process[61]. Previous research had discovered that the decomposition of monoterpenoids produced by *Myrtis communis* in the soil was accelerated during periods of high microbial activity[21,62]. Furthermore, some of these monoterpenoids can help to the pesticide degradation by biochemical reactions in contaminated soils[63].

<Figure 8.3 here>

8.5 Conclusions

The use of biopesticides is still limited due to expensive manufacturing processes, inadequate storage stability, vulnerability to environmental conditions, effectiveness issues, etc., compared to synthetic pesticides. About 75% of biopesticide products consisted of microbial biopesticides. Moreover, plant extracts and essential oil-based pesticide products could be an excellent alternative to synthetic chemicals. These natural components should be controlled in soil to determine their possible toxic effects, as well as their mobility and persistence. Previous studies determined biopesticides in soils, including cinnamaldehyde, diallyl disulfide, azadirachtin, rotenone, spinosyn A and D, nicotine, sabadine, veratridine, cevadine, deguelin, pyrethrins, limonene and its oxidation products. There must be considered that an extraction step previous to their analyses is needed to obtain a good recovery. QuEChERS was the most used extraction technique for most of these biopesticides obtaining higher recoveries than SLE, SPE and PLE. Subsequently, LC and GC coupled with MS have been the most employed analytical techniques for their determination obtaining low LOD and LOQ values.

On the other hand, adsorption and degradation are the most significant processes that can predict the fate of biopesticides in soils. Nevertheless, there is a little information about their degradation products from parent compounds and these can be more active than the original biopesticide, or their toxicity could be higher. Therefore, other analytical techniques based on high-resolution mass spectrometry (HRMS) analysers, such as TOF or Orbitrap, could be a suitable tool for a simultaneously detection of target, nontarget and unknown biopesticides by one single run. They must be used to achieve precise mass measurements of known biopesticide metabolites or transformation products through various degradation processes as well as for the identification of unknown biopesticides. Thus, the combination of efficient extraction procedures with analysis by GC and LC coupled to HRMS analysers could be a good strategy for the determination of several families of biopesticides in a short time. Finally, this type of

instrumentation will be needed to get comprehensive results when degradation studies should be developed.

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Figure Captions

Figure 8.1. Comparison of extraction procedures for the determination of biopesticides in soil samples by ultrahigh pressure LC-MS/MS. Method A: solid-liquid extraction using mechanical shaking; method B: solid-liquid extraction using sonication; method C: pressurized liquid extraction; method D: modified QuEChERS. Reproduced with permission from John Wiley and Sons (Ref. 19).

Figure 8.2. GC–MS chromatograms and mass spectrums of d-limonene and its oxidation products with their quantifier ions, confirmatory ions, and retention times. Reproduced with permission from Elsevier (Ref. 27).

Figure 8.3. Dynamic of d-limonene oxidation productions in non-autoclaved S1(A), S2(B), S3(C) at the 10 mg/kg spiked levels. Reproduced with permission from Elsevier (Ref. 27).

Table 8.1. Extraction techniques used for biopesticides determination in soils[a]

Extraction tecnhique	Biopesticide	Recovery	Description	Ref
Soxhlet	Azadirachtin A	54-79%	50 g of soil + 200 mL Extraction time: 6 h	[13]
SLE	Rotenone	95-100%	5 g of soil + 10 mL ethyl acetate Extraction time: 30 min	[14,15]
	Nicotine, sabadine, veratridine, rotenone, azadirachtin, cevadine, deguelin, spinosad, pyrethrins	1-36%	5 g of soil + 10 mL of ethyl acetate Extraction time: 30 min*: Type: rotatory agitator	[19]
		3-37%	10 g of soil + 30 mL of ethyl acetate/methanol (3:1, v/v) Extraction time: 30 min Type: sonicator	[19]
SLE (SPE)	Pyrethrins (pyrethrin I and II, cinerin I and II, and jasmolin I and II)	88.1-104%	20 g of soil + 20 mL MeCN + 3 g NaCl Extraction time: 2 min 10 mL supernatant MeCN was dried and redissolved with 1 mL acetone + n-hexane (1:9; v/v) SPE clean-up: Anhydrous Na_2SO_4 (1 g) was added into Florisil SPE cartridge (preconditioned with 10 mL n-hexane)	[16]
	Pyrethrin I and II	NP	Air-dried soil samples and biopesticide solutions at 1:5, 1:25, 1:50, and 1:100 g/mL were mixed for 24 h in rotary extractor SPE clean up: C18 cartridges of 500mg octadecyl. The cartridges were eluted with 14mL of methanol	[28]
PLE	Nicotine, sabadine, veratridine, rotenone, azadirachtin, cevadine, deguelin, spinosad, pyrethrins	3-53%	5 g of soil+ ethyl acetate/methanol (3:1, v/v)	[19]

HS-SPME	96 xenometabolites	NP	6 g of soil Temperature: 40°C Extraction time: 30 min Type of fiber coating: 50/30 µm DVB/CAR/PDMS	[24]
UAE	Rotenone	72-92%	30 g of soil + 50 mL MeCN Extraction time: 30 min Clean-up step: 2 g anhydrous sodium, 5 g florisil/activated carbon (90/10 w/w) and 2 g anhydrous sodium bottom-up. Elution with 50 mL acetone/petroleum ether (v/v, 50/50)	[27]
	Cinnamaldehyde and diallyl disulfide	70 and 61%	6 g of soil + 3 mL of ethyl acetate Extraction time: 20 min	[9]
QuEChERS	Azadirachtin, rotenone, spinosyn A and D	83-104%	5 g of soil + 5 g of water + 50 µL of internal standard solution (isoproturon-D6 at 150 µg mL^{-1}) + 100 µL acetic acid in 10 mL MeCN Extraction time: 5 min Salts and buffers: 0.5 g disodium hydrogen citrate sesquihydrate, 1 g trisodium citrate dihydrate, 4 g anhydrous magnesium sulfate, and 1 g sodium chloride Clean up step: 150 mg PSA and 150 mg C18	[18]
	Nicotine, sabadine, veratridine, rotenone, azadirachtin, cevadine, deguelin, spinosad, pyrethrins	30-110%	5 g of soil Hydration step: 2.5 mL of water was added shaken for 30 min Solvent: 5 mL MeCN with 1 % acetic acid Extraction time: 1 min	[19]

			Salts and buffers: 4 g $MgSO_4$, 4 g NaCl, 0.5 g disodium hydrogen citrate sesquihydrate, 1 g trisodium citrate dihydrate No clean-up	
	Spinosad	98-102%	10 g of soil Hydration: 7 mL of water was added and was vortexed for 25–30 min Solvent: 10 mL MeCN Extraction time: 5-6 min. Clean-up step: 1.5 mL aliquot of supernatant was transferred to a 2-mL C-18 SPE tube	[20]
	Carvone	95.7-104.2%	5 g + 5 mL of water + 10 mL n-hexane Extraction time: 2 min Salts and buffers: 2 g $MgSO_4$ and 1 g NaCl, Cean-up step: 1 mL of the supernatant was transferred to a 2.5 mL polypropylene tube containing 40 mg C18 and 100 mg Na_2SO_4	[21]
	d-limonene oxide isomers, (-)-carveol isomers, and (-)-carvone	71.2 to 114.5%	5 g of soil + 10 mL n-hexane + 5 mL of water Extraction time: 2 min. Salts and buffers: 2 g $MgSO_4$ and 1 g NaCl Extraction time: 1 min Clean-up step: 1 mL of the supernatant with 2.5 mL polypropylene tube containing 40 mg C18 and 5 mg GCB and vortexed for 2 min	[26]

[a] Abbreviations: SLE: solid liquid extraction, SPE: solid phase extractions, HS-SPME: Headspace-Solid Phase Microextraction, UAE: ultrasound assisted extractions, QuEChERS: Quick, Easy, Cheap, Effective, Rugged & Safe, NP: Not provided, PSA: Primary secondary amine, C18: octadecylsilyl, DVB: Divinyl-benzene, CAR: Carboxen, PDMS: Polydimethylsiloxane, MeCN: acetonitrile.

Table 8.2. Gas chromatography methods used for the determination of biopesticides in soils[a]

Compounds	Chromatographic conditions			Detection	LOQ	Ref
	Stationary phase	**Carrier gas/ Mobile phase**	**Other conditions**			
Azadirachtin A	O-17 column	N_2	Flow rate: 15 mL/min, Injection volume: 5 µL	FID	NP	13
Cinnamaldehyde and diallyl disulfide	HP-5MS GC column (30 m, 0.25 mm, 0.25 µm)	He	GC–MS interface, ion source, and quadrupole temperatures: 280, 230, and 150°C, respectively Flow rate: 1.0 mL/min; Injection volume: 2µL Spitless mode Total analysis time: 15.91 min	Q-MS	4.4-16 µg/kg 34-82 µg/kg	9
Pyrethrins	DB-5MS fused-silica (30 m × 0.25-mm, 0.25-µm)	He	Injector temperature: 260°C Flow rate: 1.2 mL/min; Injection volume: 2µL	MSD	50 µg/kg	16
96 xenometabolites	Agilent J&W DB-5MS GC (30 m, 0.25 mm, 0.25 µm)	He	Inlet temperature: 230°C Flow rate: 1 mL/min Splitless mode Total analysis time: 30min	EI-Q-MS	NP	24
Spinosad	G.C. Column T.R. ™ 5 MS (30 m × 0.25 mm, 0.25 µm)	He	Transfer line temperature: 280°C Injector temperature: 230ºC Flow rate: 1.3 mL/ min; Injection volume: 1µL Splitless mode	MS/MSTQD	2-13 µg/kg	20

			Total analysis time: 19.2 min			
Limonene oxide isomers, (-)-carveol isomers, and (-)-carvone	HP-5MS (60 m x 0.25 mm, 0.25 µm)	He	Inlet, MS interface, Ion source and quadrupole temperatures: 270°C, 280°C, 280°C and 150°C, respectively The split ratio was set to 5:1 Flow rate: 1.5 mL/min; Injection volume: 1 µL	Q-MS	6–48 µg/kg	[26]
Carvone	HP-5MS (60 m x 0.25 mm, 0.25 µm)	He	Ion source, quadrupole, and interface temperatures: 280°C, 150°C and 270°C The split ratio: 5:1 Flow rate was 1.5 mL/min; Injection volume: 1 µL	Q-MS	10-50 µg/Kg	[21]

[a] Abbreviations: LOQ: Limit of quantification; Q: Single quadrupole; QqQ: Triple quadrupole; MS: Mass spectrometry; MS/MS: Tandem mass spectrometry; NP: Not provided; EI: Electron impact; MSTQD: Spectrometry triple quadrupole; MSD: Mass selective detector; FID: Flame ionization detector.

Table 8.3. Liquid chromatography methods used for the determination of biopesticides in soils[a]

Compounds	Chromatographic conditions			Detection	LOQ	Ref
	Stationary phase	**Carrier gas/ Mobile phase**	**Other conditions**			
Pyrethrin I and II	Waters Radial- pak 8MBC1810 (4 µm)	MeCN and water Gradient profile	Column temperature: 25°C; Flow rate: 1.5 mL/min; Injection volume: 100 µL	UV (λ = 230 nm)	15-25 ng	[28]
Rotenone	Acclaim C18 reverse (150 mm x 4.6 mm, 5 µm)	MeCN and water Isocratic (60:40 v/v)	Flow rate: 1.0 mL/min; Injection volume: 20 µL	UV (λ = 295 nm)	NP	[14]
	Waters XTerra C18 (250 mm x 4.6 mm, 5 µm)	MeCN and water Isocratic (60:40 v/v)	Flow rate: 1.0 mL/min; Injection volume: 100 µL	DAD (λ = 295 nm)	NP	[14]
	Waters XTerra MS RP_{18} (250 mm x 2.1 mm, 5 µm)	MeCN and water (0.1 % TFA) Isocratic (60:40 v/v)	Flow rate: 0.4 mL/min; Injection volume: 20 µL	Q-MS (ESI)	15 µg/kg	[14]
	XDB (250 mm x 2.1 mm, 5 µm)	MeCN and water Isocratic (75:25 v/v)	Flow rate: 0.4 mL/min; Injection volume: 20 µL	QqQ-MS/MS (ESI)	NP	[15]
	Zorbax TC-C18 (250 mm x 4.6 mm, 5 µm)	MeCN and water Isocratic (70:30 v/v)	Flow rate: 1.0 mL/min; Injection volume: 10 µL	UV (λ = 299 nm)	20.3 µg/kg	[27]
Azadirachtin, rotenone, spinosyn A and D	BEH C18 (100 mm x 2.1 mm, 1.7 µm)	Water and MeOH with 0.1% ammonium acetate. Gradient profile	Column temperature: 30°C; Flow rate: 0.3 mL/min; Injection volume: 5 µL	QqQ-MS/MS (ESI)	6-9 µg/kg	[18]
Nicotine, sabadine, veratridine, rotenone,	BEH C18 (100 mm x 2.1 mm, 1.7 µm)	MeOH and aqueous solution of ammonium	Column temperature: 30°C; Flow rate: 0.3 mL/min;	QqQ-MS/MS (ESI)	1-10 µg/kg	[19]

azadirachtin, cevadine, deguelin, spinosad, pyrethrins	formate (5 mM). Gradient profile	Injection volume: 5 µL

[a] Abbreviations: MeCN: Acetonitrile; DAD: Diode array detector; ESI: Electrospray ionization; LOQ: Limit of quantification; MeOH: Methanol; Q: Single quadrupole; QqQ: Triple quadrupole; MS: Mass spectrometry; MS/MS: Tandem mass spectrometry; NP: Not provided; UV: Ultraviolet; MSTQD: Spectrometry triple quadrupole; TFA: Trifluoroacetic acid.

Table 8.4. Physical and chemical properties of biopesticides

Compound	**K_{oc} (L/kg)**	**DT_{50} (days)**	**Ref**
Rotenone	10000	5 hours-2.76	[14,27]
Azadirachtin A	20.6–875.1	19.8-43.9	[42,43]
Azadirachtin B	Not available	20.8-59.2	[42,43]
Spinosyn A	35024	6.5-46.3	[48,49]
Spinosyn D	Not available	11.3–62.6	[48]
Sabadine	1.8×10^5	Not available	[64]
Veratridine	2.3×10^6	Not available	[64]
Cevadine	9.7×10^4	Not available	[64]
Sabadinine	6.1×10^4	Not available	[64]
Nicotine	100	< 0.5	[53]
Pyrethrin I	26915	1.8	[56]
Pyrethrin II	2042	73.2	[56]
Cinerin I	9332	2.7	[56]
Cinerin II	700	97.2	[56]
Jasmolin I	21380	1.9	[56]
Jasmolin II	1622	36.8	[56]
Carvone	111	0.2-5	[59]

Figure 8.1. Comparison of extraction procedures for the determination of biopesticides in soil samples by ultrahigh pressure LC-MS/MS. Method A: solid-liquid extraction using mechanical shaking; method B: solid-liquid extraction using sonication; method C: pressurized liquid extraction; method D: modified QuEChERS. Reproduced with permission from John Wiley and Sons (Ref. 19).

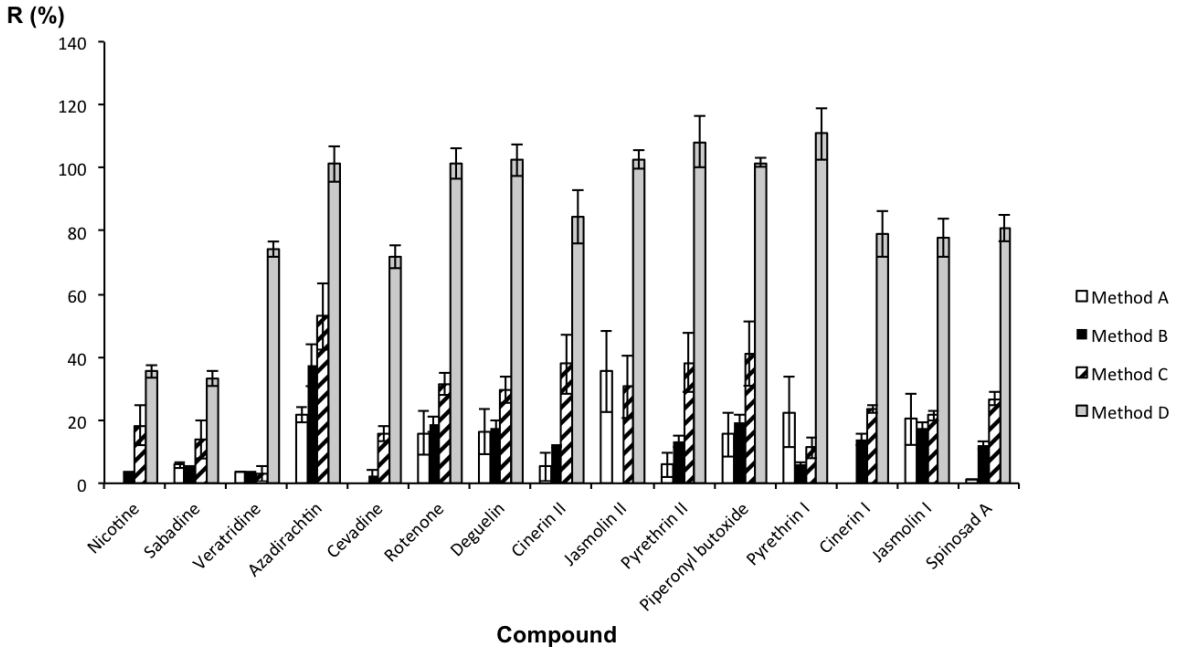

Figure 8.2. GC–MS chromatograms and mass spectrums of d-limonene and its oxidation products with their quantifier ions, confirmatory ions, and retention times. Reproduced with permission from Elsevier (Ref. 27).

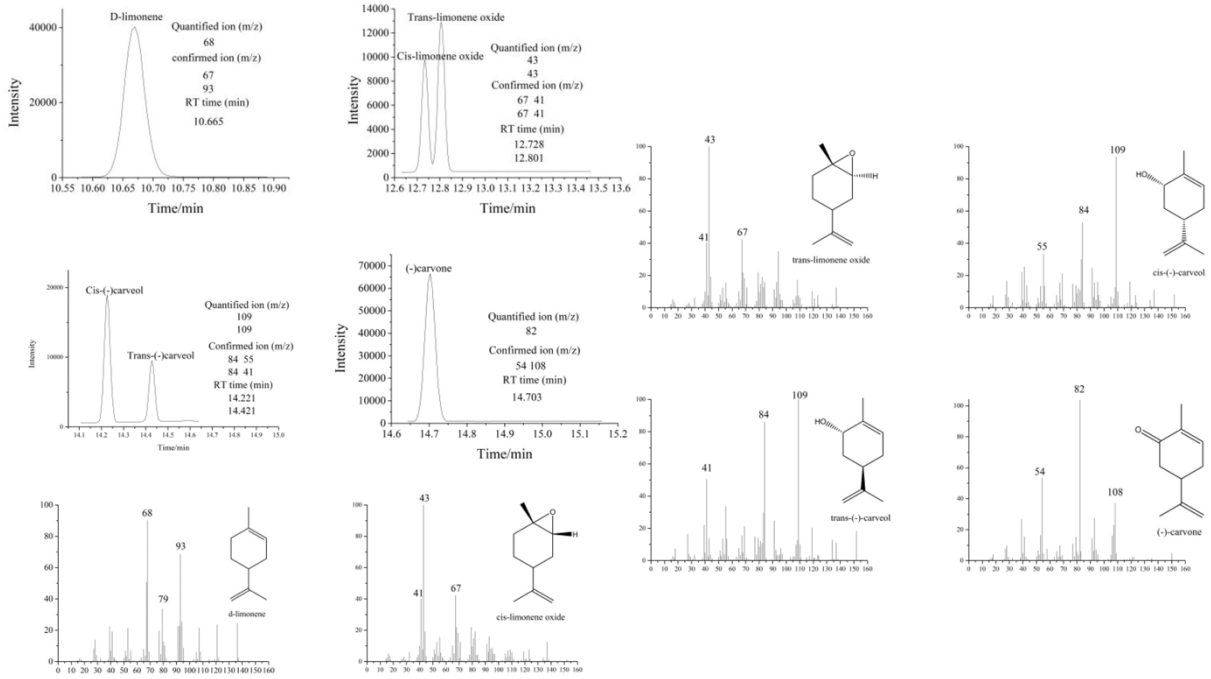

Figure 8.3. Dynamic of d-limonene oxidation productions in non-autoclaved S1(A), S2(B), S3(C) at the 10 mg/kg spiked levels. Reproduced with permission from Elsevier (Ref. 27).