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Review

Analysis of the Environmental Impact of Botanical Pesticides in Soil

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Abstract: Plant-based pesticides are considered viable complements of conventional synthetic pesticides in agriculture. Their environmentally benign nature and potential to mitigate ecological impacts render them advantageous options for sustainable farming practices. However, the long-term effects of botanical pesticides on soil ecosystems remain unclear. This review aims to examine current evidence concerning the persistence of botanical pesticides in soil environments and their potential effects. Specifically, it addresses their biodegradation pathways in soil as well as their impact on soil enzymes and biology. The methodologies available to perform these studies are also briefly discussed, particularly focusing on how they can be tailored to improve the analysis of the impacts and challenges posed by the use of botanical pesticides in ecosystems.

Keywords: biodegradation; botanical pesticide; non-target organisms; soil analysis; soil enzymes

1. Introduction

The term soil health can be understood as the capacity of soil to function as a vital and supportive ecosystem for all organisms [1,2]. To a large extent, ecosystem degradation has highlighted the importance of monitoring soil health, especially in agricultural soils subjected to intense farming and the excessive use of agrochemicals, such as pesticides [3]. Given the increasing knowledge of the hazardous effects of several pesticides on the environment and human health, policies surrounding their use across the globe have undergone significant changes over the years. For example, the European Commission has outlined a milestone to reduce the use of conventional pesticides by 50% by 2030, with the current usage already at 320,000 tons in 2022 [4–6]. These policy changes have also alerted consumers to prefer certified organic and sustainable farming products, driving higher investments in the research, development, and formulation of botanical pesticides. In response, farmers are adapting their agricultural practices to meet these demands, showing a preference for botanical pesticides in crop protection because plant-based extracts and essential oils tend to be cheaper and more accessible than microbial pesticides and plant-incorporated protectants [7]. Consequently, the commercialization of biopesticides has increased over the years, being projected to expand at a Compound Annual Growth Rate of 9.47% between 2023 and 2030, potentially reaching a market value of USD 30 billion [8]. Nevertheless, EU Regulation No. 1107/2009, together with the Directive 2009/128 on the sustainable use of pesticides, presents barriers for the registration and commercialization of botanical pesticides, as can be appreciated in more detail in specific publications [5,9,10]. Botanical pesticides should have appropriate registration guidelines that are different

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from other pesticides for several reasons. Unlike many traditional pesticides that are synthetized through chemical reactions in a laboratory, botanical pesticides are obtained from biological sources through processing and, therefore, cannot be registered by the same standards. Special attention must also be given to the conditions under which the source plants grow because factors such as geographical areas and climatic conditions influence their qualitative and quantitative composition. This may result in some variability in their profiles, which synthesized compounds do not experience. Additionally, the term "botanical pesticide" is used to describe a wide range of products, ranging from a single plant-derived compound to an extract or a fraction of an extract and even a plant powder. Establishing a proper registration system for these agrochemicals will help to reduce misinformation or a lack of information and speed up their commercialization [9]. For this reason, the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) published the International Code of Conduct on Pesticide Management to provide specific guidelines for the registration of different pest control agents for plant protection and public health use, including botanical pesticides [11].

The use of plants to deter pests came from ancient civilizations, such as the Romans, using *Veratrum viride* Aiton as a rodenticide and pyrethrum as an insecticide in Persia and Dalmatia [12]. Moreover, food waste has been proposed as an alternative source for the research and extraction of botanical pesticides in edible and/or endemic plants because of its high abundance, easy availability, and global need to reduce the ecological footprint associated with food waste [13,14].

Botanical pesticides can exert a variety of biopesticidal activities because of their wide range of metabolites (Figure 1), of which alkaloids, phenolics, and terpenes are the most abundant. True alkaloids can be synthesized through the shikimate pathway using the amino acid tryptophan as a precursor and/or the acetyl-CoA biosynthesis pathway using ornithine and lysine as precursors [15]. These compounds exhibit broad-spectrum activity, and some well-known examples of their biopesticidal properties include nicotine (commonly used as an insecticide) [16], berberine (noted for its antifungal activity) [17], and sanguinarine (recognized for its nematicidal activity) [18]. Phenolic compounds are secondary metabolites synthesized via the shikimate and phenylpropanoid pathways. This large family includes molecules with one aromatic ring (such as phenolic acids and hydrolysable tannins) or more rings (such as flavonoids, condensed tannins, stilbenes, and lignans). Phenolics with notable biopesticidal activity include tannic acid [19], rotenone [20], and naringenin [21–23]. Other active compounds, like the terpenes, known for their volatile and lipophilic nature, are derived from the mevalonate pathway. Monoterpenes are highly abundant in essential oils and are responsible for their insecticidal activity, because they interfere with crucial physiological and biochemical processes. For instance, López and Pascual-Villalobos [24] verified that the monoterpenes fenchone, *S*-carvone, and linalool inhibit acetylcholinesterase activity. One popular terpene derivative is azadirachtin, extracted from *Azadirachta indica* seeds and leaves, which belongs to the nortriterpenoid group and arises from the oxidation and degradation of tetracyclic triterpene precursors [25,26]. In addition to these three large chemical families, pyrethrins are an important group of botanical pesticides. They were first identified between 1750 and 1880, were typically isolated from *Chrysanthemum cinerariaefolium*, and are categorized in pyrethrin I (includes pyrethrin I, cinerin I, and jasmolin I) and pyrethrin II (includes pyrethrin II, cinerin II, and jasmolin II) [16]. However, owing to their low stability under varying climatic conditions, synthetic modifications of their chemical skeletons have led to the development of widely used pyrethroids. Other bioactive molecules such as saponins [27], polyketides [28,29], and fatty acids [30] can be found in plant-based extracts and essential oils.

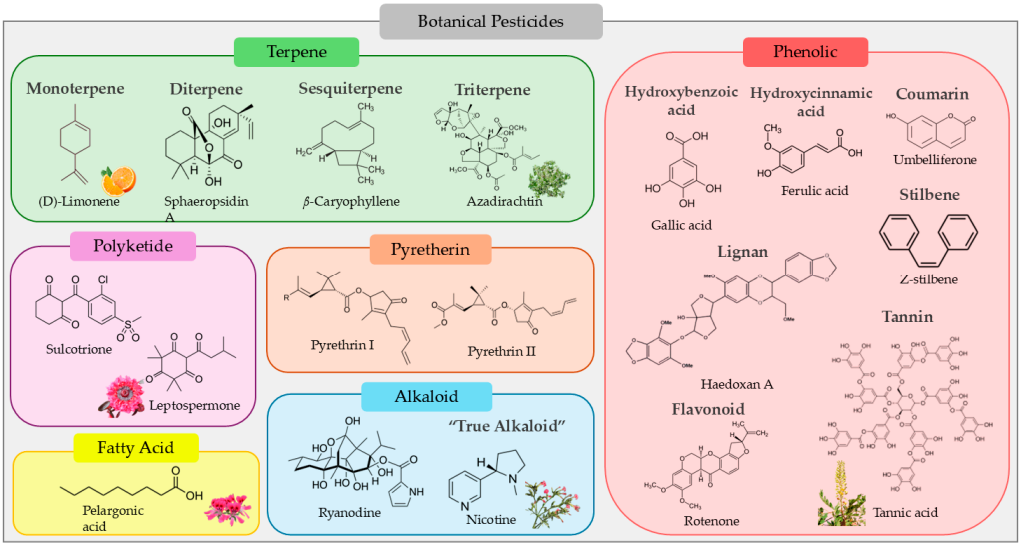


Figure 1. Main metabolite classes found in botanical pesticides.

There is a widespread perception that botanical pesticides, due to their natural origin, are inherently safe to (micro)organisms, are rapidly degraded under abiotic conditions, and show low persistence in soil. Although they offer lower environmental and health risks compared with synthetic pesticides, this assumption does not translate directly into reality. For example, the isoflavone rotenone, a broad-spectrum botanical insecticide with acaricidal properties, is non-selective, and minimum doses can induce Parkinson's disease in rats by triggering the progressive death of dopaminergic neurons, α-synuclein inclusions, and microtubule destabilization [31–33]. Moreover, the persistence of rotenone and its primary degradation product, rotenolone, in soil has been shown to be dependent on temperature and soil type [34]. Table 1 provides an overview of other botanical pesticides with major drawbacks, which resulted in the prohibition of some of them in the European Union (EU), the United States of America (USA), and other countries. Therefore, the unintended effects of botanical pesticides should not be overlooked, along with the persistence of secondary metabolites originating from different biodegradation routes.

Table 1. Modes of action and drawbacks of popular botanical pesticides.

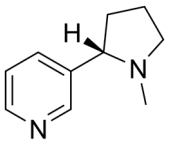
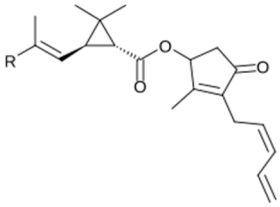
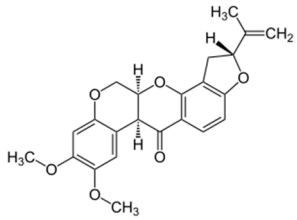
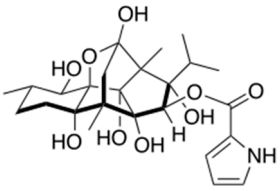
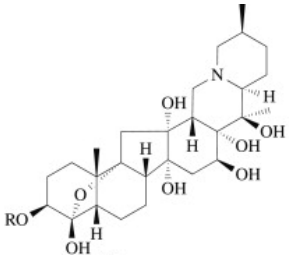
Botanical Pesticide	Mode of Action	Drawbacks	Reference
Nicotine sulfate (from *Nicotiana tabacum* leaves) 	Disrupts the insect's nervous system, resulting in death due to convulsions and/or paralysis.	Highly toxic to mammals by inhalation and skin contact, to blooded animals and other insects.	[16]
Pyrethrins (from *Chrysanthemum* genus) 	Act on sodium channels of axonal membranes by altering their permeability, resulting in a decrease of sodium and potassium effluxes. The physiological effects are excitation, lack of coordination, and paralysis. They can also inhibit ATPases.	The pyrethrum crude extract may cause diverse complications in humans. Toxic to bees and fish.	[16,35,36]

Table 1. *Cont.*

Botanical Pesticide	Mode of Action	Drawbacks	Reference
Rotenone (from *Derris*, *Lonchocarpus*, and *Tephrosia* genus) 	Forms a complex with NADH dehydrogenase, inhibiting the oxidation of NADH to NAD and therefore, blocks the oxidation by NAD of several substrates. Inhibits mitochondrial electron transport and mitosis.	Toxic to mammals by inhalation, ingestion, and skin contact. Induces Parkinson's disease in rats. Highly toxic to fish.	[16,32,33,36]
Ryanodine (from *Ryania speciosa*) 	Acts at the level of the sarcoplasmic reticulum membrane by binding to ryanodine receptors in muscle cells, leading to insect muscle contractions or paralysis depending on the concentration.	Produces irreversible contractures in vertebrate skeletal muscle and negative inotropic responses in mammalian cardiac muscle.	[37–39]
Veratrine (mixture of alkaloids from *Schoenocaulon officinale*) 	Affects the nerve cells, resulting in loss of nerve function, paralysis, and death. Acts as a contact and stomach poison to insects.	Irritating to humans if inhaled and by skin contact.	[16]

This review aims to summarise the current knowledge on the effects and overall impact of botanical pesticides on soil ecosystems. For this purpose, the techniques of detection and extraction used to study the dynamics of these molecules in soil are presented, along with a brief description of their decomposition profile. A summary of the main research findings on the influence of botanical pesticides on soil biology and biochemical processes is presented.

2. Degradation Rate, Metabolites, and Routes

Table 2 shows the decomposition profiles of some botanical pesticides in different soil types. The persistence of a pesticide in soil can be predicted by knowing its half-life, processes that lead to its decomposition, and the respective degradation products. These aspects are dictated by soil physicochemical characteristics, indicating that the same pesticide can exhibit different degradation rates in distinct soils [40]. For example, the degradation of botanical pesticides may occur at a slower rate in soils rich in clay and organic matter, as evidenced by Cavoski et al. [31] for rotenone and Liu et al. [41] for thymol, due to their adsorption properties. These molecules are also less susceptible to hydrolysis at low pH values, leading to a higher persistence in soil. This fact has been shown to limit, for instance, the degradation of carvone [42] and salicylic acid [43] (Table 2). In the latter case, the degradation was also dependent on the degree of moisture and temperature, being slower at lower temperatures [43]. Moisture can facilitate contact between pesticides and decomposers due to diffusion and mass flow, whereas temperature

can affect the intensity of hydrolysis and photodegradation [44]. Interestingly, salicylic acid degradation was independent of salt concentration in the soil, a parameter known to interfere with microbial activity and that could compromise the acid degradation by microorganisms [45]. Another factor that can limit the degradation of botanical pesticides is soil depth. Similarly to the traditional pesticides, botanic pesticides are prone to percolate through the soil layers, reaching deeper layers. Under these conditions, photolysis and microbial-mediated degradation are compromised due to the lack of light penetration and microbial activity [46].

Table 2. Description of the decomposition profiles of some botanical pesticides in different soil types.

Botanical Pesticide	Soil Type/Texture	Decomposition Profile	Reference
Essential Oils (EOs)			
Cinnamomum verum	Sandy clay and clay loams	– *trans*-cinnamaldehyde was the main component and was rapidly degraded; – Possible degradation metabolites were hydroxycinnamic acids.	[47]
Citrus sinensis	Sandy clay and clay loams	– *D*-Limonene was the main component and was rapidly degraded; – Possible degradation metabolites were thymol, cymene, isoterpinolene, and cymenene.	[47]
Mentha piperita	Fallow land	– Reduction of 90% of the initial concentration after 30 days; – After 60 days, monoterpenoids concentration fell from around 90% to 20% and the sesquiterpenes concentration increased; – *β*-caryophyllene concentration increased more than 15-fold.	[48]
Mentha spicata	Fallow land	– Reduction of 90% of the initial concentration after 30 days; – After 60 days, monoterpenoids concentration fell from around 90% to 45% and the sesquiterpenes concentration increased; – *β*-caryophyllene concentration increased more than 15-fold.	[48]
Rosmarinus officinalis	Fallow land	– Reduction of 50% of the initial concentration after 30 days; – Monoterpenes were highly abundant; – High persistence in soil after one year following its application.	[48]
Plant-Based Extracts			
Cortaderia speciosa	Experimental field	– Continuous decrease overtime in concentration of six phenolic acids (ferulic acid, *p*-coumaric acid, caffeic acid, vanillic acid, *p*-hydroxybenzoic acid, and gallic acid); – *p*-hydroxybenzoic acid (target metabolite) displayed a $t_{1/2}$ of approximately 31 h.	[49]

Table 2. *Cont.*

Botanical Pesticide	Soil Type/Texture	Decomposition Profile	Reference
Pure/Isolated Compounds			
Arbutin	Scrub soil of *Polygonella* shrubs	– Degradation was faster in nonsterile soil; – Rapid degradation, reaching nondetectable levels after 2 days; – Hydroquinone was the main metabolite originated from degradation.	[50]
Benzoic acid	Bulk soil	– $t_{1/2}$ = 2.57 days.	[51]
Benzoquinone	Scrub soil of *Polygonella* shrubs	– Degradation was faster in nonsterile soil; – High persistence.	[50]
Biochanin A	Sandy loam	– Degradation products: dihydrobiochanin A, pratensein, genistein (predominant); – DT_{90} < 20 days.	[52]
Carvone	Agricultural soil	– *S*-enantiomer degraded faster than *R*-enantiomer; – Acidic soils retarded carvone degradation and lowered enantioselectivity.	[42]
	Agricultural soil	– *S*-carvone degradation rate was similar when applied as a free compound or granules in soil.	[53]
Cinnamic acid	Bulk soil	– $t_{1/2}$ = 1.29 days.	[51]
2,4-Dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one	Wheat crop soil	– Degradation products: 7-methoxy-2-benzoxazolinone ($t_{1/2}$ = 31 ± 1 h), 2-hydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one, 2-amino-7-methoxyphenoxazin-3-one ($t_{1/2}$ = 5 ± 1 days).	[54]
Formononetin	Modified agricultural soil	– Biodegradation proceeded after a lag phase of around 24 h with a concentration reduction around 4.5% after 72 h when compared to control.	[55]
Gallic acid	Scrub soil of *Polygonella* shrubs	– Degradation was faster in nonsterile soil; – Rapid degradation, reaching nondetectable levels after 3 days.	[50]
Hydroquinone	Scrub soil of *Polygonella* shrubs	– Degradation was faster in nonsterile soil; – High persistence in soil; – Benzoquinone was one of the metabolites originated from degradation.	[50]
p-hydroxybenzoic acid	Bulk soil	– $t_{1/2}$ = 4.22 days.	[51]

Table 2. *Cont.*

Botanical Pesticide	Soil Type/Texture	Decomposition Profile	Reference
Pure/Isolated Compounds			
4-hydroxy-(2H)-1,4 benzoxazin-3(4H)-one	Cultivated crop soil	– No degradation in sterile soil; – $t_{1/2}$ = 3.6 ± 0.2 days; – After 48 h, only one degradation metabolite was detected.	[56]
Limonene	Silty, silty clay, and sandy clay loams	– Degradation products: predominantly *trans*-compound isomers (*trans*-limonene oxide and *trans*-(-)-carvoel) with concentration variations depending on soil type; – Faster degradation in sandy clay loam.	[57]
Luteolin	Experimental field	– Degradation fitted the first-order model; – $t_{1/2}$ was higher under high concentrations (10 and 40 $\mu g g^{-1}$ of dry soil).	[58]
Naringenin	Modified agricultural soil	– Biodegradation occurred without a detectable lag phase but with concentrations reduced to approximately 0.13 $\mu g g^{-1}$ from 50 $\mu g g^{-1}$.	[55]
Pulegone	Agricultural soil	– Higher dissipation in non-sterilized soils; – *R*-enantiomer degraded faster than *S*-enantiomer in acid soils.	[59]
Salicylic acid	Sandy loam	– Temperature, soil humidity, and pH affected the degradation rate, while salt concentration had no effect; – In a 3-day experiment, degradation was lower at 4 °C and pH = 3.5–5.3 when compared to control; – The presence of mercury chloride in soil completely inhibited the degradation when compared to non-sterilized soil.	[43]
Scopoletin	Sandy, clay, and sandy clay loams	– Slow degradation in sterilized soils; – High $t_{1/2}$ in a non-sterilized sandy loam soil but with a reversible sorption.	[60]
Umbelliferone	Sandy and clay loams	– After 7 days of incubation, the residual percentage in two non-sterilized sandy loams decreased to 10 and 0%, while in the sterilized controls it remained at over 75%.	[61]
	Calcic Cambisol soil	– After 1 day, the concentration in unamended soil decreased from 25 to 15 $mg kg^{-1}$. After that, the concentration remained stable.	[62]
Vanillin acid	Bulk soil	– $t_{1/2}$ = 1.56 days.	[51]

The existing literature indicates that microbial-mediated processes represent the predominant degradation pathway for botanical pesticides in soils. Numerous studies have observed accelerated degradation in non-sterilized soils (Table 2), suggesting that soil microorganisms expedite the decomposition of botanical pesticides by utilizing them as carbon sources for energy production [42,50,56,59,60]. Botanical pesticides are also subject to abiotic degradation processes, such as photolysis and hydrolysis, although these are typically investigated in aqueous media rather than in soil. Cavoski et al. [31] reported a rapid decrease in the concentration of rotenone in thin-layer soil plates after five hours of direct sunlight exposure, followed by a gradual decline over time.

Although a fair amount of research has been conducted on the pesticidal activity of botanical pesticides, especially essential oils, the identification and persistence of the secondary metabolites originating from abiotic and biotic degradation processes remain poorly explored. Recently, Reyes-Ávilla et al. [47] proposed a simple pathway for trans-cinnamaldehyde degradation in soil based on the identification of its secondary metabolites. The authors hypothesized that reactions of oxidation, aldehyde reduction, olefinic epoxidation, and dihydroxylations were responsible for the degradation of trans-cinnamaldehyde in the identified products. Mączka et al. [63] proposed a biodegradation pathway for carvacrol based on the literature revision and showed that the degradation metabolites identified depended heavily on the microorganism involved in their metabolization. This knowledge is highly important because the degradation products can be more persistent than the original applied substances, as observed by Mácias et al. [54] (Table 2), and show pesticidal activity, especially to non-target organisms, compromising soil health in the short and long term. Regardless of the importance of this knowledge, the traditional research employed in laboratories to obtain these answers is time-consuming and expensive. As a result, researchers have dedicated their efforts to developing efficient and accurate prediction methods and algorithms to evaluate the biodegradation, persistence, and toxicity of pollutants [64–66], including pesticides such as cypermethrin [67]. For instance, Jenner et al. [68] performed a persistency screening assessment of eleven cyclic sesquiterpenes commonly found in essential oils using a standard test for ready biodegradability (Organization for Economic Co-operation and Development 301F Manometric Respirometry test) and prediction models (BioWinTM, BioHCwin, and Catalogic). The models used were considered of limited use because the studied sesquiterpenes did not fit within the structural domain of the models; however, the authors concluded that these compounds were not persistent and would be expected to degrade under environmental conditions. In another study, the transformation products and pathway of guvermectin in different soils were investigated and revealed the involvement of hydrolytic, redox, and deamination reactions that produced small organic acids, inosine or adenosine analogues. The authors predicted very low acute and chronic toxicity for guvermectin metabolites against fish species by using the predictive model Ecological Structure Activity Relationships (ECOSAR) [69].

3. Impact in Soil Biology and Biochemistry

Soil biology, size, and diversity directly indicate the capacity of soil as a living and dynamic system that supports plant growth and nutrient cycling [70]. These organisms include nitrogen-fixing bacteria, mycorrhizal fungi, and invertebrate animals, including earthworms and beetles. Each of these biological communities has crucial roles in the soil, ranging from participation in organic matter degradation to improving soil structure and fertility. Given their high importance in maintaining normal soil functions, it is critical that (bio)pesticides do not cause harm to these organisms or reduce their physiological functions in soil.

a. Effects on Microorganisms

Beneficial microorganisms are responsible for regulating soil properties and fertility. They participate in organic matter decomposition and transformation, resulting in carbon cycling and fixation in the soil. Additionally, they facilitate interactions between plants and soil by acting as intermediators, a role particularly attributed to arbuscular mycorrhizal fungi [71–73]. Considering that biopesticides, including botanical pesticides, often lack target specificity, it is important to assess how these molecules or organisms affect the natural soil microbiome in a positive, negative, direct, or indirect manner. For instance, Xie et al. [74] observed that the wuyiencin-based biopesticide produced by *Streptomyces ahygroscopicus* var. *Wuyiensis* modulates the native bacterial community in the rhizosphere of tomato plants.

The literature reveals that the effects of botanical pesticides on soil microorganisms are diverse, ranging from stimulation to the inhibition of microbial growth. Jouni et al. [75] studied the variations induced by the three essential oils in the populations of soil microorganisms. The authors verified that, except for *Thymbra capitata* EO at the highest concentration, the essential oils disturbed the bacterial and fungal populations, but the soils recovered their initial functionalities over time. At variable concentrations, *Mentha piperita* EO led to an initial decrease in fungi and bacteria, which was more consistent for Gram-positive bacteria, but they reached normal levels when compared to the control at the end of the study. The effect of *Santolina chamaecyparissus* EO was dependent on the applied concentration, especially for fungi, as bacteria were not significantly affected. In another study, the impact of carvacrol and thymol on microorganisms in four different types of agricultural soil was investigated [76]. The authors verified that these monoterpenes exhibited dose- and time-dependent effects on microbial enzymes, with more pronounced effects for carvacrol, which led to the inhibition of enzyme activity. The negative effects of dihydrochalcone, isoflavone, aliphatic phenol, and spinosad on soil bacterial and fungal populations have been observed in one of the three studied soils in a dose-dependent manner [77].

In addition to the direct evaluation of the pesticidal activity of molecules against soil microorganisms, some studies have analyzed variations in certain soil biochemical parameters or indicators related to microbial activity. This was the case for Jouni et al. [75], who monitored changes in extractable carbon, microbial mass carbon and nitrogen, cumulative carbon dioxide, and respiration rate. The most interesting result was obtained for *T. capitata* EO at the highest concentration, which decreased the microbial mass carbon and nitrogen, increased extractable carbon, and decreased the respiration rate compared to the control by the end of the study. These observations implied that, at this dose, *T. capitata* EO killed some of the soil microorganisms, and these negative effects occurred after two months. *T. capitata* is rich in carvacrol, which is frequently used as fungicide and nematicide, aiming to control phytopathogenic microorganisms. This study emphasizes the care that must be taken in the use of botanical pesticides since beneficial microorganisms can also be affected.

Recent trends in pesticide formulations have focused on their encapsulation to overcome their non-target specificity. Polysaccharides have been widely advocated as encapsulating materials because they present high versatility in functional groups, which can be chemically modified to improve mechanical strength and better control of pesticide release [78]. Moreover, they are widely available in nature and are, in general, biodegradable, biocompatible, non-toxic, and cheap. However, these materials alone can exhibit antimicrobial activity and can have synergistic or antagonistic effects when combined with botanical pesticides. Recently, Chmiel et al. [79] demonstrated that the amount of maltodextrin used for the encapsulation of peppermint and caraway EOs influences their activity against soil microorganisms. Peppermint EO with high concentrations of maltodextrin stimulated bacterial growth in several soil types, whereas it promoted inhibition at lower concentrations.

At low maltodextrin concentrations, fungal growth was reduced when compared to the control, whereas, at higher concentrations, fungal growth was not affected in sandy soil. On the other hand, caraway oil encapsulated in high amounts of maltodextrin inhibited bacterial growth in sandy soil and stimulated actinomycete growth, while, at lower doses, no effect was observed on bacteria and inhibitory effects were detected on actinomycetes.

b. Effects on Arthropods

Arthropods are largely responsible for maintaining biological equilibrium in soil by fragmenting and/or humidifying organic matter and by altering the soil structure, hydrology, and mineral and organic matter compositions [80]. These invertebrate species include nematodes, annelids, enchytraeids, earthworms, snails, and slugs.

Earthworms are the most studied non-target soil arthropods for botanical pesticides because they are believed to be the most important organisms in agriculture. Their functions in soil include the improvement in microbial activity in decaying organic matter, the spread of soil microorganisms, the protection of soil organic matter in aggregates, the maintenance of soil structure, and an increase in water retention [80,81]. Bernardi et al. [82] verified that spinosad, a biologically derived insecticide, was metabolized by *Eisenia fetida* (Savigny, 1826) without significantly compromising their health, and that DNA damage only occurred significantly by the end of the toxicity test, revealing a safer ecotoxicological profile than the organophosphate chlorpyrifos. Another study reported that the leaf oil of *Piper betle* (L.) presented LC_{50} values of 3149 and 4081 mg/kg to *Eudrilus eugeniae* (Kinberg, 1866) and *E. fetida*, respectively, did not affect their development rate after 14 days, and attracted them to soil containing leaf oil [83]. Vivekanandhan et al. [84] demonstrated that *Melaleuca cajuputi* EO, studied as a candidate for biological control of the malarial vector, did not exhibit toxicity against *E. eugeniae*. *Ocimum sanctum* L. EO lacked toxicity against *E. fetida* [85], and *Spheranthus amaranthroids* EO was considered harmless against *E. eugeniae* at the highest concentrations [86].

Non-target toxicity assays involving beetles are mostly focused on species from the Coccinellidae family, which are widely known for their predatory activity against insects of the order Homoptera and mites [87]. Lami et al. [88] evaluated the direct exposure toxicity of five insecticides against the beetle *Propylea quatuordecimpunctata* (L.) (Linnaeus, 1758). These five products were Prev-Am® Plus (*C. sinensis* EO as the active ingredient), Cerrus®, Aglio (crude *Allium sativum* L. extract); Rabona® (pyrethrins), EcorNaturaSi® (Marseille soap), and an insecticide with *Thymus vulgaris* L. EO as the main ingredient. The authors verified that the susceptibility of adults and larvae to insecticides was different, especially for the pyrethrin-based insecticide, which induced significantly higher mortality in the larval stage than in the control. EO-based products and *A. sativum* extract had no effect on adult beetles. In the larval stage, the *C. sinensis* EO had a comparable mortality to the pyrethrin-based insecticide, whereas the *T. vulgaris* EO and *A. sativum* extracts did not significantly affect mortality compared to the control.

To a lesser extent, green lacewings have also been used as model organisms in toxicity assays. The ethanolic extracts of *Ficus carica* branches and leaves (at 0.21 mg/cm^2) did not affect the beetles *Coleomegilla maculata* (De Geer, 1775) and *Eriopis connexa* (Germar, 1824) [89]. In another study, the EO of *Ocotea indecora* leaves did not induce mortality in *E. conexa* and *Chrysoperla externa* (Hagen, 1861) at 2.20 µL/mL, which was the concentration capable to kill 95% of *Drosophila suzukii* (Matsumura, 1931) flies [90].

Mites belonging to the Phytoseiidae family have been evaluated as non-target arthropods for botanical pesticides, given their role as biological control agents in many crop cultures [91]. For example, Duso et al. [92] studied the effects of three botanical pesticides, including spinosad, on the mite species characteristic of vineyards, particularly *Typhlodromus pyri* (Scheuten, 1857), *Amblyseius andersoni* (Chant, 1957), and *Phytoseius finitimus*

(Ribaga, 1904). The authors observed a decrease in the population of these mite species. In another study, five botanical pesticides were screened for toxicity against *Phytoseiulus persimilis* (Athias-Henriot, 1957) and *Amblyseius swirskii* (Athias-Henriot, 1962) [93]. Requiem®, an extract of *Chenopodium ambrosioides*, showed low mortality (<25%) against *P. persimilis* at the recommended field dose. Prev-Am®, an extract of *Citrus aurantium*, and FLiPPER®, an olive oil extract, caused moderate mortality in *P. persimilis* (>25%). Prev-am® and FLiPPER® at the recommended field dose resulted in moderate mortality (>25%) of *A. swirskii*.

Parasitoids and spiders were among the other arthropods studied. In *Camellia sinensis* trees, matrine and azadirachtin had no significant effect on coccinellids, spiders, and parasitoids [94]. Lami et al. [88] verified that none of the tested insecticides affected the parasitoid *Aphidius colemani* (Viereck, 1912).

c. Impact in Soil Enzymes

Soil enzymes are catalytic proteins that play a central role in nutrient cycles (including carbon, nitrogen, phosphorous, sulfur, and others) and constitute an important marker of soil health. Mainly secreted by soil microorganisms and plants, the most common enzymes in soil are oxidoreductases, such as dehydrogenase and catalase, and hydrolases, like urease and amylase [95,96].

As expected, terpenes dominate the studies that have assessed the effect of botanical pesticides on soil enzymes, as they are the main class of compounds found in EOs. For instance, Adamczyk et al. [97] examined the possible changes induced by monoterpenes (α-pinene, carene, and myrcene), diterpenes (abietic acid and colophony), and triterpenes (β-sitosterol) in the enzymatic activity of five enzymes crucial for nutrient cycling (β-glucosidase, chitinase, protease, acid phosphatase, and arylsulfatase). In vitro studies have indicated that all terpenes are potential inhibitors of the tested enzymes. Additional experiments to evaluate the inhibitory effects of terpenes against chitinase and β-glucosidase were performed in soil samples differing in nitrogen and organic matter content. The results showed a decrease in the activity of both enzymes but with a less clear pattern of inhibition, probably due to soil heterogeneity and complexity. As a result, this study highlights the importance of not relying solely on in vitro studies to characterize the effects of pesticides on soil enzymes. Papatheodorou et al. [98] analyzed the effect of carvone enantiomers on the enzymes of agricultural soil involved in the carbon, phosphor, and nitrogen cycles. For urease, the authors found that the variation in enzyme activity, either inhibition or stimulation, was clearly enantioselective. This hydrolase activity was inhibited by *S*-carvone but was initially enhanced and later inhibited by *R*-carvone. Regarding alkaline phosphomonoesterase, no effect was observed for *S*-carvone; however, changes in activity were detected for *R*-carvone. Campolo et al. [99] evaluated the side effects of two citrus EO formulations in the soil enzymatic activities of one oxidoreductase and three hydrolases, namely dehydrogenase and alkaline phosphomonoesterase, acid phosphomonoesterase, and urease, respectively. The authors verified that none of the tested formulations, EOs, or carriers had an impact on normal soil enzymatic activity. In another study, the effects of encapsulated mixtures of geraniol and eugenol and geraniol and cinnamaldehyde on the activity of three enzymes were assessed [100]. Arylsulfatase and β-1,4-glucosidase activities remained equal with increasing doses of the encapsulated mixtures, whereas acid phosphatase exhibited high sensitivity to the formulations applied at the highest dosage (100 mg kg^{-1}) after 2 weeks of exposure. Recently, it was reported that carvacrol and thymol showed dose- and time-dependent activities against dehydrogenase and N-acetylglucosaminidase in vineyard soils [76]. No inhibition was detected for these two monoterpenes against acid phosphatase, leucine aminopeptidase, or β-glucosidase.

Little is known about the possible inhibitory effects of polyphenolic compounds, which are the main components of plant-based extracts. Tannins dominate phenol re-

search with this goal because of their numerous functions, including plant defense and the inhibition of microbial activity [101]. However, their impact on soil enzymes is usually studied from the perspective of application as soil amendments rather than as botanical pesticides. Adamczyk et al. [101] evaluated the effect of tannins on four enzymes: acid phosphatase, arylsulfatase, β-glucosidase, and chitinase. In vitro studies have shown that tannins act as noncompetitive inhibitors of all enzymes at high concentrations, whereas at low concentrations, they enhance catalytic activity by increasing the enzyme's coiled structures. Moreover, these polyphenols have a low affinity for β-glucosidase. In the soil experiments, the authors added condensed tannins to soils with high and low contents of several enzymes (acid phosphatase, β-glucosidase, arylsulfatase and chitinase) and observed that the addition of medium and high concentrations of tannins to soils inhibited enzyme activity, while at lower concentrations, the opposite effect was detected. Later, Liu et al. [102] verified that the addition of condensed tannins to soil enhanced the catalytic activity of soil enzymes involved in nitrogen cycling, particularly amidase, N-acetyl-β-d-glycosaminidase, polyphenol oxidase, peroxidase, urease, and laccase. The authors hypothesized that these polyphenols were likely carbon substrates for soil microorganisms because they also detected higher soil respiration rates with the addition of tannin. Other polyphenols have been the subject of research, such as flavonoids. For example, the isoflavonoid formononetin had no effect on dehydrogenase activity, while the flavanone naringenin had an overall but non-systematic impact [55].

The short-term effects of five saponins (hederagenin, bayogenin, medicagenic acid, zanhic acid, and soyasapogenol B) from *Medicago sativa* on four soil hydrolases (β-glucosidase, leucine aminopeptidase, alkaline phosphomonoesterase, and arylsulfatase) from seven soil samples with different origins and properties were studied by Tava et al. [103]. Saponin's effect on enzyme activity was revealed to be dependent on the soil physicochemical characteristics, the applied concentration, and the type of enzyme. For example, saponins enhanced β-glucosidase activity in only two samples, namely a sandy-loam soil sample with a high carbon content from a meadow and a sandy-loam soil sample from a greenhouse. In contrast, high concentrations of saponins decreased the activity of alkaline phosphomonoesterase in four soil samples. Additionally, saponins reduced microbial biomass when 10 and 20 mg/g of soil in the saponin mixture was added to soil; however, the overall microbial enzymatic activities were less affected than the microbial biomass. This observation could be the result of an adaptive response of soil microbial communities to the presence of these molecules.

Our research on the non-target effects of botanical pesticides in soil biology and biochemistry reveals the scarce and sometimes contradictory knowledge surrounding these aspects. There are several knowledge gaps that must be filled in order to assess the true impact of botanical pesticides on soil. Some of these gaps result from the lack of representation and diversity of potential target organisms (for example, organisms other than earthworms) and/or contradictory studies that do not allow for the generalization of the impact of these pesticides, namely, whether they stimulate or inhibit microbial growth and soil enzymes (Figure 2).

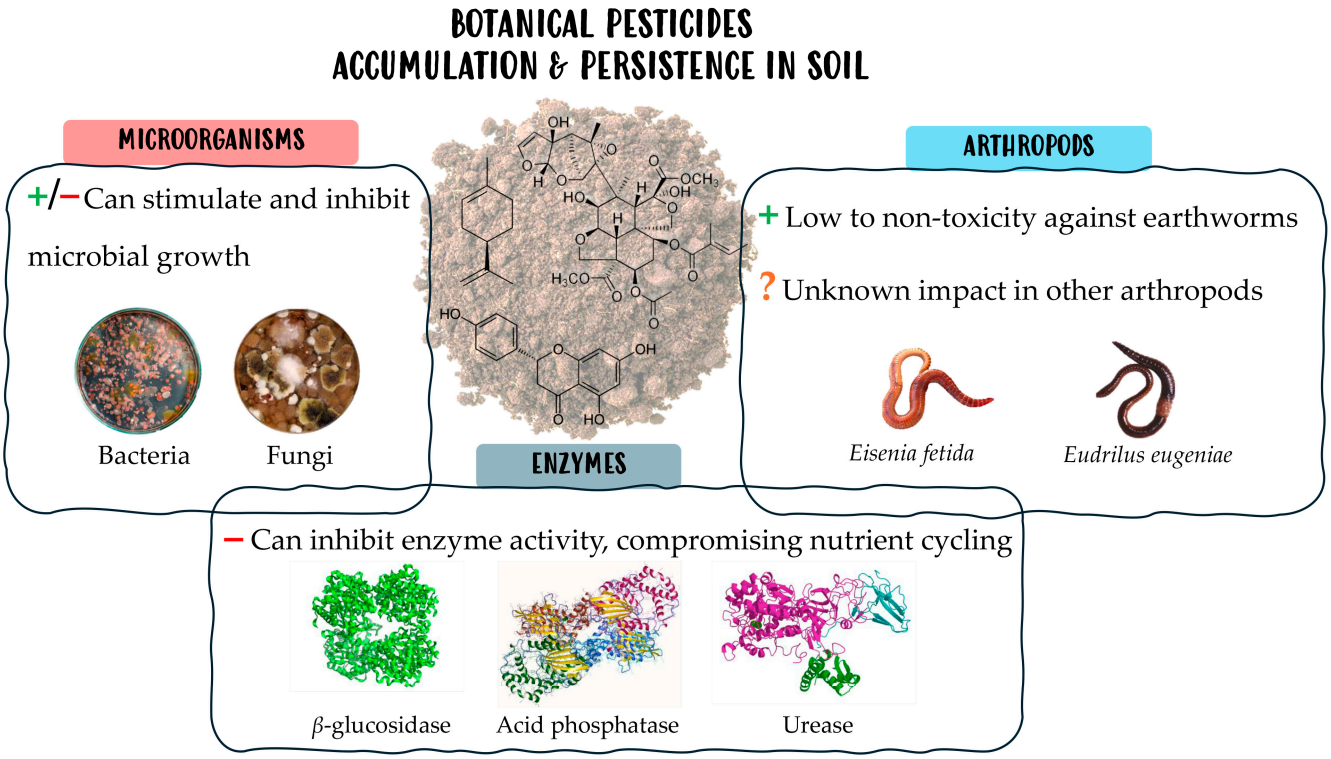


Figure 2. Overview of the effects of botanical pesticides accumulation and persistence in soil on microorganisms, arthropods and enzymes.

4. Analysis of Botanical Pesticides in Soils

A significant challenge in the field of botanical pesticide research lies in the complex process of extracting and analyzing these substances from soil. The unique properties of both the botanical pesticides and the soil matrix must be carefully considered when selecting appropriate extraction and analytical methods to ensure accurate results. Soil samples are complex and heterogeneous, with many organic and inorganic interferents, preventing the selective extraction of compounds. Moreover, these compounds are often present in low amounts and have a short lifespan, being sensitive to fluctuations in temperature and humidity, which affects their stability and persistence in soils [104,105]. Botanical pesticides are chemically diverse, which prevents the development of universal and comprehensive methods for their extraction and analysis [105]. Overall, there is a lack of specific and standardized analytical methods for botanical pesticide analysis in soil samples, which must be urgently improved (Table 3).

Table 3. Methodology applied for analysis of botanical pesticides in different soil types.

Botanical Pesticide	Soil Type (Amount)	Methodology	LODs/LOQs (µg/kg)	Rec (%)	Ref
Arbutin, gallic acid, benzoquinone, hydroquinone	Scrub soil (sandy) of *Polygonella* shrubs	SLE: 1 g sieved air-dried soil sample (1.5 mm); 2 mL water extraction (60 min, 3× vortex); brief centrifugation, 0.2 µm nylon filtration; HPLC-UV	1 (µg g^{-1})	---	[50]
Biochanin A	Sandy loam	ASE: 15 g pulverized dried sample poured into a 33-mL extraction cell, diluted to 50 mL with 70% MeOH (5 min preheat, 5 min heat, 3 min static; flush, 80%; purge, 60 s; cycles, 4; 1500 psi; 40 °C); HPLC-MS/DAD	---	---	[48]
Carvone	Sand, sandy clay loam, silty loam and loam	QuEChERS: 5 g sample + 10 mL n-hexane + 5 mL water (50 mL tube); 2 min vortex; 2 g $MgSO_4$ + 1 g NaCl; 1 min vortex; 7000 rpm 5 min; 1 mL supernatant + 40 mg C18 + 100 mg Na_2SO_4 (2.5 mL tube); 2 min vortex; 5000 rpm 5 min; 0.5 mL supernatant filtration (0.2 µm); GC-MS	10–50	94.4–97.9	[106]
Cinnamaldehyde and diallyl disulfide	fine sand, sandy loam, and silty clay loam soils	UAE: 6 g soil + 3 mL EtOAC, 1 min vortex, 20 min UAE extraction (RT); 1 min vortex, 3000 rpm 10 min; filtrate supernatant; repeat extraction twice; pool supernatants; GC-MS	15–83 (ng g^{-1})	61–70	[107]
Limonene & trans-cinnamaldehyde	Sandy clay loam and clay loams (5 g)	SLE: soil samples + IS were shaken with 10 mL EtOAC (1 h), 5 min 5000 rpm, filtered and injected; GC-HRMS or UHPLC-HRMS	1–10	83–106	[44]
Limonene	Silty, silty clay, and sandy clay loams	QuEChERS: 5 g air-dried sieved (0.9-mm) sample; 10 mL n-hexane + 5 mL water (50 mL tube); 2 min vortex; 2 g $MgSO_4$ + 1 g NaCl, 1 min vortex; 5 min 7000 rpm; 1 mL supernatant + 40 mg C18 + 5 mg GCB; 2 min vortex, 5 min 5000 rpm; 0.5 mL supernatant filtration (0.2 µm filter); GC-MS	1–16/4–48	71–114	[50]
Matrine and Berberine	Farming areas (China)	SLE: 10 g sample + 1 mL standard solution (50 mL tube), 1 min vortex; 30 min resting; 1% ammonia–ACN (20 mL), 5 min 1200 strokes/min shaker; 2 g NaCl, 1 min vortex; 5 min 3500 rpm; 2 mL upper layer + 25 mg PSA (PTFE centrifuge tube); vortex, 5 min 3500 rpm; supernatant filtration (0.22 µm); UHPLC-MS/MS	0.34–1.07/1.12–3.58	73.1–109.3	[108]

Table 3. *Cont.*

Botanical Pesticide	Soil Type (Amount)	Methodology	LODs/LOQs (μg/kg)	Rec (%)	Ref
Matrine	Farming areas (China)	QuEChERS: 10 g soil + 2 mL 25% ammonia (50 mL tube); vortex, rest 10 min; 20 mL ACN, 3 min vortex; 1 g NaCl + 4 g anhydrous $MgSO_4$; 1 min vortex; 5000 rpm 5 min; 1.5 mL supernatant + 100 mg PSA + 100 mg anhydrous $MgSO_4$ (2 mL tube), 1 min vortex; 12,000 rpm 2 min; filter supernatant (0.22 μm); HPLC-MS/MS	5–10	74.4–98.4	[109]
Phenolic acids (p-coumaric acid, ferulic acid, vanillic acid, caffeic acid, p-hydroxybenzoic acid, and gallic acid)	Experimental fields (Catholic University of Córdoba)	SLE: MeOH extraction (3 × 50 mL); solvent evaporation; HPLC-UV	---	---	[46]
Phenolic acids (p-hydroxybenzoic, acid, benzoic acid, cinnamic acid, and vanillin acid	Bulk soil	SLE: 5 g air-dried, sieved (2-mm) and grounded soil samples; extractions with Mehlich III solution + NaOH (25 g sample) at 25 °C (24 h incubation); 10 min 4000 rpm; supernatant pH adjustment (2.5); 0.22 μm filtration; HPLC-UV	---		[47]
peppermint, spearmint and rosemary leaf EOs	Fallow land	Hydrodistillation (Clevenger apparatus, 3 h); GC-MC (1 μL injection)	---	---	[45]
Pyrethrins (pyrethrin I and II, cinerin I and II, and jasmolin I and II)	Farming areas (China)	SLE: 20 g sample + 20 mL ACN + 3 g NaCl, 2 min vortex mixer extraction (50-mL tubes); 5 min 3800 rpm; 10 mL supernatant (100-mL flask) rotary concentrated almost-dry state (one drop of liquid), dried (N_2 steam); SPE cleanup: residue redissolved 1 mL acetone + n-hexane (1 + 9; v + v) loaded in activated SPE cartridge (1 g anhydrous Na_2SO_4 conditioned with 10 mL n-hexane); 10 mL acetone + n-hexane (1 + 9; v + v) elution; evaporation to dryness; 1 mL acetone redissolution, 0.22 μm filtration; GC-MS	12–24/50	88.1–104	[110]

Table 3. *Cont.*

Botanical Pesticide	Soil Type (Amount)	Methodology	LODs/LOQs (µg/kg)	Rec (%)	Ref
Spinosad	Sandy loam	SLE: 25 g sample + 25 mL acetone (250 mL conical flask), 10 min horizontal shaker; supernatant filtration (Whatman No. 42 filter paper); repeat thrice; extract clean-up with liquid-liquid partitioning (3 × 30 mL DCM); pooled DCM evaporation (rotary evaporator); HPLC-UV	50	80–82	[111]
	Farming areas (China)	SLE: 20 g sample + 5 mL water + 40 mL ACN (100 mL tube); 1 min vortex; + 7 g NaCl, 1 min vortex, 10 min 4000 rpm; 20 mL supernatant concentrated to dryness (vacuum rotary evaporator); + 2 mL ACN/EtOAC (3:1, *v*/*v*); SPE: ACN/EtOAC (3:1, *v*/*v*) preconditioning (5 mL) and elution (25 mL); eluent evaporation (N_2 stream); redissolution (2 mL MeOH); filtration (0.22 µm); UHPLC-MS/MS	1/5	83.4–85.3	[112]
	aridisol and entisol along with saline and calcareous soils	QuEChERS: 10 g soil + 7 mL water (50 mL); 25–30 min vortex; 10 mL ACN extraction (5–6 min); 1.5 mL supernatant clean-up (2 mL C-18 SPE tube), 2 min vortex, 2 min centrifugation (≥5000 g); GC-MS/MS	---	98–102%	[113]
Myrica gale methanolic extract	Silt loam soil	SPME: Soil samples homogenized, passed through a 2 mm sieve, dark storage 4 °C); 6 g soil (crimped 20 mL HS-SPME vials) + 7.2 mg dry Myrica gale methanolic extract; automatic HS-SPME: 50/30 µm DVB/CAR/PDMS fibre, 5 min incubation, 30 min extraction, at 40 °C; GC-MS	---	---	[114]
2,4-Dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one	Cultivated crop soil	US MeOH extraction (10 mL, 15 min, 5 °C); 5 min, 13000 rpm; repeat using EtOAC as extraction solvent (10 mL); combine extracts, distillation (reduced pressure); 2 mL MeOH + 1% acetic acid dissolution; filtration; HPLC-UV	---	---	[54,56]

ACN—acetonitrile; ASE—accelerated solvent extraction; DCM—dichloromethane; EtOAC—ethyl acetate; GC-MS/MS—gas chromatography coupled with tandem mass spectrometry; HPLC-MS/DAD—high-pressure liquid chromatography-mass spectrometry-diode-array detection; HRMS—high resolution mass spectrometry; HS-SPME—headspace solid-phase extraction; LOD—limit of detection, LOQ—limit of quantification; QuEChERS—Quick, Easy, Cheap, Effective, Rugged, and Safe; RT—room temperature; SLE—solid-liquid extraction; SPE—solid-phase extraction; UAE—ultrasound-assisted extraction; US—ultrasound bath; UV—ultraviolet detection.

The techniques established for analyzing synthetic pesticides are the reference points for the analysis of pesticides of botanical origin in soil. Accordingly, it is worthwhile to refer to the recent comparative study of diverse techniques to detect pesticide residues in soil under the principles of Green Analytical Chemistry, cost, and time [115]. This research points to QuEChERS, pressurized fluid extraction, ultrasound-assisted extraction (UAE), and solid–liquid partitioning as widely utilized extraction procedures in soil sample extraction [115]. Among those, the QuEChERS technique, an acronym for Quick, Easy, Cheap, Effective, Rugged, and Safe, has emerged as one of the most environmentally friendly and sustainable methods for this type of analysis. Its popularity stems from several inherent benefits, including rapid sample preparation, limited use of harmful chemicals and solvents, straightforward application, and cost-effectiveness [115–117]. Regarding analysis, various techniques can be employed depending on the nature of the pesticide. GC with nitrogen/phosphorus detection, LC with diode array detection, and mass spectrometry are commonly utilized for chemical analysis, although LC-MS has a superior capacity for analyzing a broader range of pesticide residue chemical classes, especially compounds with poor volatility [115]. Focusing our literature query specifically on the extraction of botanic pesticides from soils (Table 3), QuEChERS is undoubtedly a popular methodology [57,106,109,113]. Nevertheless, solid–liquid extractions with water [48,50] and organic solvents [47,49,51] using rotary evaporation [110–112] or distillation [54,56] have also been reported. The use of ultrasounds [54,56,107] or high pressure [48] further improves the efficiency of the extraction procedure, although additional equipment is required. The work reported by Prestes et al. [118], in 2012, is a hallmark in the determination of botanical pesticides in agricultural soils. In this work, the authors assayed various extraction techniques, such as solid–liquid extraction with mechanical agitation, ultrasonication, and pressurized liquid extraction, to extract several botanical pesticides, namely nicotine, sabadine, veratridine, rotenone, azadirachtin, cevadine, deguelin, spynosad D, and pyrethrins. Overall, QuEChERS extraction, without cleanup steps, followed by ultra-high-pressure liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS), was reported as the most effective approach [118]. Moreover, by skipping the clean steps of the extraction step, the authors considerably increased the greener profile in terms of time, use of dispersive reagents and labware, and waste. Solid-phase microextraction (SPME) is one of the most successful extraction procedures in analytical chemistry and is particularly tailored for the analysis of volatile and semi-volatile compounds. As a proof of concept, Ghosson, Raviglione, Salvia and Bertrand [114] applied online Headspace-Solid-Phase Microextraction–Gas Chromatography–Mass Spectrometry-based untargeted metabolomics to characterize the volatile composition of *Myrica gale* methanolic extract and its dynamics over 38 days after its application in the soil. The *methanolic extract of M. gale* is known for its herbicidal properties, but its composition, effects, and fate are not completely understood. This non-destructive and cost-effective approach using only 35 min HS-SPME-automated extraction and a 36 min GC-MS run allowed for the identification of 63 herbicide compounds for the first time. These include the six major compounds, Eucalyptol, L-terpinen-4-ol, α-terpineol, α-terpineol acetate, 3,7(11)-selinadiene and Germacrone, with the remaining predominantly terpenes, aromatic and aliphatic esters, alcohols and ketones [114]. An overview of the main experimental approaches currently used for the analysis of botanical pesticides in soils is given in Figure 3.

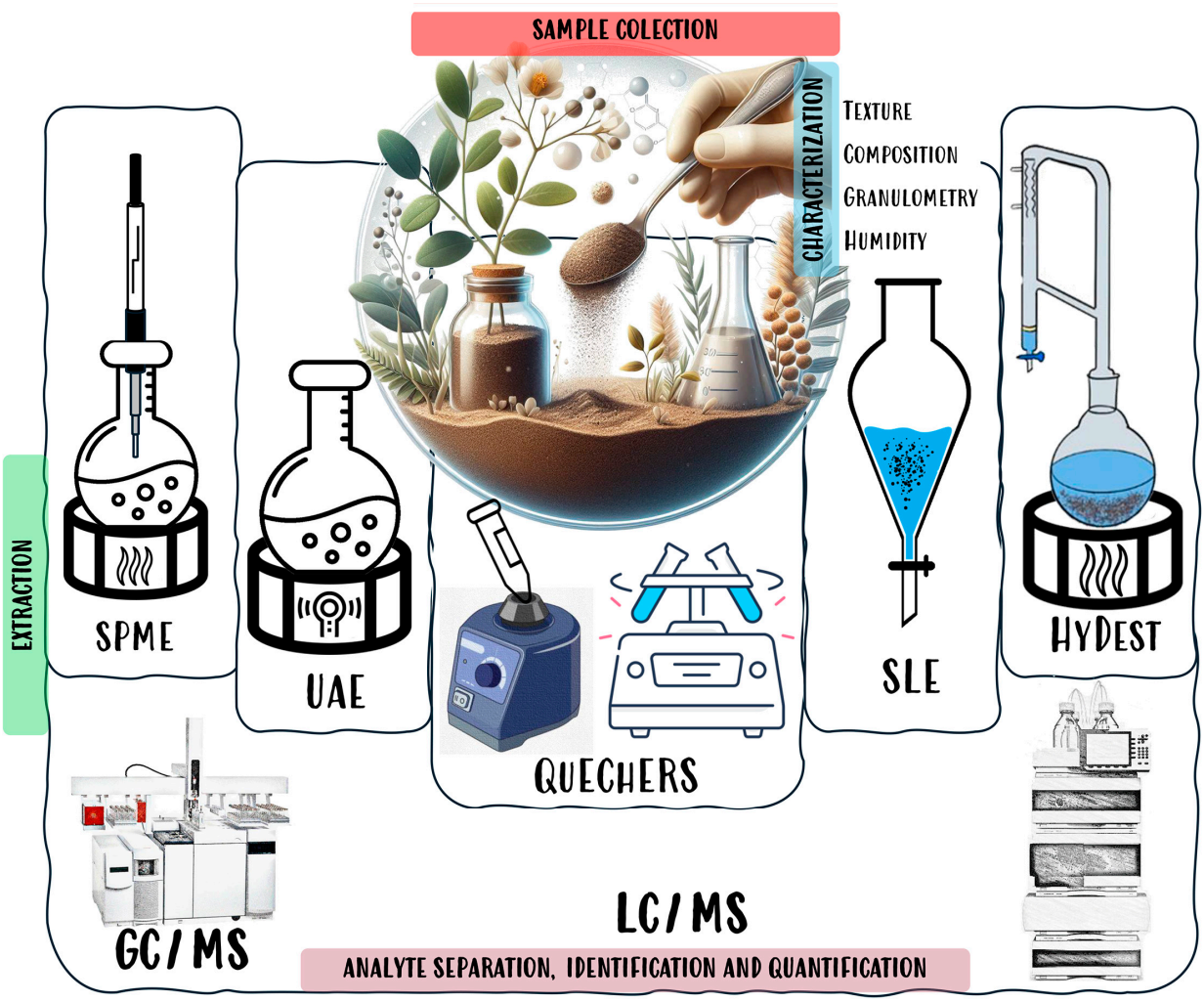


Figure 3. Overview of the most common experimental layout involving soil samples analysis for botanical pesticides presence and quantification. Legend: GC-MS—gas chromatography mass spectrometry; HyDest—Hydrodistillation; LC-MS—liquid chromatography mass spectrometry; SPME—solid-phase extraction; QuEChERS—Quick, Easy, Cheap, Effective, Rugged, and Safe; SLE—solid–liquid extraction; UAE—ultrasound-assisted extraction.

5. Conclusions and Future Directions

Botanical pesticides are aligned with the current trends in modern agriculture and are becoming increasingly important in pesticide research and innovation. However, obstacles prevent widespread biopesticide production, including limited resources, complex manufacturing processes, and high natural resource utilization [31]. Botanical pesticides are mainly used as complements, acting synergistically to lower the dosage of the synthetic pesticides required to obtain the same activity, and are characterized by limited efficacy and selectivity. Botanical pesticides should target harmful pathogens while minimizing effects on soil beneficial microorganisms and processes, but most studies focus on pest and pathogen control, ignoring their non-target effects. Moreover, the studies that investigate potential side effects in soil organisms and enzymes reveal that botanical pesticides have variable effects, being hard to predict if they can negatively impact soil normal functions. By protecting soil natural processes and organisms, selective botanical pesticides support long-term soil health and sustainable agriculture [119], having fewer unintended effects on non-target organisms and ecosystems compared with broad-spectrum synthetic pesticides [120]. This highlights the need for comprehensive research on the selectivity of botanical pesticides.

The degradation process of botanical pesticides in the soil and the resulting metabolites are not well understood. This knowledge gap is significant, as breakdown products can exhibit varying effectiveness against plant pathogens and non-target organisms compared with the original pesticide. Studying the degradation pathway of botanical pesticides will help avoid the accumulation and persistence of potentially or equally bioactive metabolites in soil, thus preventing unwanted short- and long-term effects. Although earthworms are crucial in organic matter decomposition, it is vital to consider the possible adverse effects of botanical pesticides on other soil-dwelling creatures. Beetles, which are important for pest control and soil turnover, have been proposed as indicators of environmental changes, highlighting the need to assess the impact of botanical pesticides on their populations [121]. The improvement in predictive models for biodegradation, toxicity, and pesticide persistence along with the recent rapid development of synthetic biotechnology has created opportunities to address these limitations in biopesticide development and use [122] and can be important tools to clarify the overall impact of botanical pesticides and their degradation metabolites in soil biology and biochemistry.

Concerning the analysis of botanical pesticide in soils, emerging technologies are creating more precise, rapid, and mobile methods for detecting pesticide residues, with potential applications in botanical pesticides. This includes different biosensor classes, such as enzyme-based biosensors measuring enzyme activity or immunosensors based on antigen–antibody interactions, both offering rapid and sensitive quantitative analysis (reviewed in [123]). Spectrometric sensors with molecularly imprinted polymer (MIP) films capable of detecting minute pesticide residues have been proposed as cost-effective platforms for custom pesticide screening [124]. Multi-analyte lateral flow assay (LFA) platforms capable of detecting multiple pesticides within a single sample matrix have also been developed [125]. A sensing system that uses machine learning to detect and classify pesticide levels in soil runoff samples was proposed by Dhamu et al. [126]. This device can identify both polar and nonpolar pesticides, categorizing their concentrations as low, medium, or high using artificial intelligence methods. Surface-enhanced Raman spectroscopy (SERS) is a promising technique for detecting pesticide residues on fruit and vegetable surfaces, especially when involving nanomaterials to amplify Raman signals (reviewed in [123]). Recently, Lin et al. [127] devised a novel imaging technique using a cellulose hydrogel film embedded with silver to identify pesticide residues on fruit surfaces and detect contaminants at minimal concentrations.

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