Review Article

Essential Oils in Stored Product Insect Pest Control

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Among botanical extracts used as insecticides, essential oils (EOs) are promising alternatives to chemical insecticides. EOs are synthesized by plants, and they play a key role in plant signaling processes including also attractiveness toward pollinators and beneficial insects. Plant species producing essential oils (over 17,000 species) are called aromatic plants and are distributed worldwide. Our review aims to evaluate research studies published in the last 15 years concerning the use of EOs in stored product protection. More than 50% of the retrieved manuscripts have been published by authors from Eastern countries (Iran, China, India, and Pakistan), investigating different aspects related to insect pest management (exposure route, effect on the target pest, and mode of action). Coleoptera was the most studied insect order (85.41%) followed by Lepidoptera (11.49%), whereas few studies targeted new emerging pests (e.g., Psocoptera). Almost all the trials were carried out under laboratory conditions, while no experiments were conducted under real operating conditions. Future research studies concerning the use of EOs as insecticides should focus on the development of insecticide formulations which could be successfully applied to different production realities.

1. Introduction

The ecotoxicological, environmental, and social consequences of the widespread use of chemical insecticides in agriculture have led researchers to find viable alternatives that are more environmentally friendly than synthetic chemicals. In this context, the use of insecticides based on botanical extracts is attracting considerable interest both among researchers and consumers. Among botanical extracts used as insecticides, essential oils (EOs) are a promising alternative because of their worldwide availability and relative cost-effectiveness.

Essential oils are secondary metabolites synthesized by plants, and they play very important roles in plant defense (both against biotic and abiotic stresses) and signaling processes, including also the attraction of pollinators and beneficial insects [1–4].

EOs are synthesized by plants both internally (secretory glands allocated inside the plants) as well as externally (secretory glands placed on the plant surface) [5]. They are produced by different plant organs such as flowers, herbs, buds, leaves, fruit, twigs, bark, seeds, wood, rhizomes, and roots and can be accumulated in specific histological structures (glandular trichomes, secretory cavities, and resin ducts) [6, 7]. Plant species that produce essential oils are called aromatic plants and are distributed worldwide; these plants (over 17,000 species) belong to a limited number of families: Asteraceae, Cupressaceae, Lamiaceae, Lauraceae, Rutaceae, Myrtaceae, Piperaceae, and Poaceae [5, 8].

Essential oils are mainly constituted by monoterpenes and sesquiterpenes synthesized in the cytoplasm and plastids. All terpenes are synthesized via either the methylerythritol 4-phosphate (MEP) pathway or the mevalonate-dependent (MVA) pathway. Two (C₅) isoprene precursors, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), are involved in the terpene synthesis and the isoprene units determine their class (monoterpenes, C₁₀; sesquiterpenes, C₁₅) [9]. Sesquiterpenes contain 15 carbon atoms, and they are less volatile and have a higher boiling point than monoterpenes. As a consequence, fewer of them contribute to the fragrance of EOs [10].
EOs are constituted by a blend of 20 to 70 organic compounds, some of which represent more than 80% of the constituents as append, e.g., in Sweet Orange EO, the main compound, limonene reaches 88–97% of the whole oil [11, 12]. Generally, the main components characterize the biological activity of the EOs.

EOs are hydrophobic and generally lipophilic, their density is often lower than that of water, and they are soluble in organic solvents.

Despite the numerous extraction methods used to obtain EOs from natural raw plant material, only four methods, such as (i) hydrodistillation, (ii) steam distillation, (iii) dry distillation, and (iv) mechanical processes, are considered in the European Pharmacopoeia and the International Standard Organization on Essential oils (ISO 9235:2013) [4]. The EO can undergo physical treatments, which do not result in any significant change in its composition (e.g., filtration, decantation, and centrifugation). The resulted products consist of a blend of volatile compounds with a strong odor [13].

1.1. Hydrodistillation. This method is considered the simplest one to obtain EOs from the plant material by immersion of biomass in boiling water [10]. The oil contained in the oil cells diffuses by means of osmosis in the hot water; then the steam, produced by boiling water, carries the oil vapors in a condenser. The condensed EOs are separated from water by decantation.

1.2. Steam Distillation. In the steam distillation, the vapor is supplied in such a way that liquid water does not come into contact with the vegetal raw material. In the simplest version, steam is generated by water added in the lower part of the distiller; the plant raw material is separated from the liquid water by a perforated grid. The steam that passes through the plant material carries the oil vapors, and after passing through a condenser, EO is separated from water by decantation.

1.3. Dry Distillation. This technique involves heating the plant material in the absence of oxygen, which would promote combustion, and without adding water or steam. This method is not commonly used. The EOs produced using dry distillation are cade and birch. Rectification is often necessary to remove undesirable molecules that may have formed.

1.4. Mechanical Process. The mechanical process, also known as cold-pressing method, consists in extracting EOs at ambient temperature without involving heat [10]. This method is used for the production of Citrus spp. and Fortunella spp. peel oils.

In addition to those previously described, other extraction methods are developed with the aim to improve the quality, the yield, and to decrease the energy consumption (i.e., solvent extraction, microwave-assisted extraction, ultrasonic extraction, Soxhlet extraction, subcritical or superheated water extraction (SCWE), and supercritical fluid extraction).

2. EOs against Stored Product Insects

Although some reviews on the potential of essential oils as repellents and/or insecticides have been published, there is no critical review about their use in stored products protection. One of the most important characteristics of essential oils, their phytotoxicity, may favor their use as herbicides, but at the same time limit their use in crop protection [14, 15]. Stored product sector seems to be a perfect candidate for the development of new EO-based alternative pest control strategies.

The aim of this review was to analyze research studies on the use of essential oils in stored product protection (sensu lato) as carried out in the last 15 years. The scientometric analysis of publications on EOs against stored product pests was based on documents retrieved from the Scopus database (see Supplementary Materials for Supplementary Method 1).

In the last 15 years, 210 documents were published (Figure 1). Among the 210 publications, 197 are articles, 9 book chapters, 3 reviews, and 1 conference paper. The three retrieved reviews deal with general aspects of the use of essential oils such as green pesticides against a range of insects, including few stored product pests [16], related to few plant families [17], or applied only as fumigants [18].

These studies were published by researchers of 47 countries distributed worldwide, but almost the 50% of the retrieved studies were published by researchers from Iran (21.63%), China (17.14%), India (5.30%), and Turkey (5.30%) (Figure 2).

The EOs used in the various experiments were extracted mainly from aerial parts (71.88%), with leaves (28.51%) as the major EO source material. The other plant materials used for the EO extraction were resin, gum, rhizomes, and roots. In 22.49% of the trials, the EO sources were not reported. Hydrodistillation was the most widely used extraction method (52.91%) followed by steam distillation (8.25%). Many analysed papers (29.12%) report neither the extraction method used for the EOs production nor the part of the plant processed, probably due to the use of commercial oils. In the various experiments carried out, 65.18% of the EOs were chemically characterized, whereas in 30% of the trials, this information was missing. Furthermore, book chapters and review papers, as well as papers aimed to evaluate just the activity of single components of some EOs, provide no information about the chemical characterization of the mentioned EOs.

The retrieved studies investigate several topics related to the control of stored product insects (Figure 3). Since many retrieved papers address different insecticidal activity (contact toxicity, fumigation, mode of action, etc.) and/or different essential oils, our data analysis, unless otherwise specified, refers to single trials (EO—target species—effect).

Among those aimed at killing the insect pests, 44.21% of the trials regard the use of essential oils, or EO-based insecticide formulations, applied as fumigants; 21.66% evaluated the contact toxicity, and less than 1% of the studies
tested the insecticidal efficacy by ingestion route. Other investigated aspects were the mode of action of EOs (2.48%) or the effects of these natural compounds on the life history traits of insects (6.72%).

Coleoptera were the most studied insect order (85.41%) with the two key species *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* (L) (Coleoptera: Curculionidae) which accounted for almost 50% of the coleopteran studies. Lepidoptera were used in 11.49% percent of the trials, in which *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) and *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) represented almost all the studies carried out against this insect order. The remaining studies concerned aspects related to Psocoptera control, and *Liposcelis bostrychophila* (Badonnel) (Psocoptera: Liposcelididae) was used in almost all studies involving this insect order (29 out of 35 trials) (Figure 4).

### 3. Insecticidal Activity

A huge number of research studies aimed at assessing the insecticidal activity of EOs against crop pests as well as...
against disease vectors [1, 19], but less attention has been paid to stored product pests. Here, we briefly review the results achieved using EO treatments against stored product pests according to their application method (i.e., contact, fumigation, and ingestion) and insecticidal activity (i.e., toxicity and repellence). In detail, we empathize the most promising results for every insect family, highlighting, when possible, similarities or divergences between pest and/or EO plant species. Furthermore, studies aimed at evaluating modifications of EOs activity attributable to geographic origin, EO-based formulations, synergism with other insecticidal compounds, and research studies reporting characteristic results were reported.

4. Contact and Ingestion Toxicity

In the last decade, several research studies focused on the insecticidal activity of EOs through contact and ingestion routes (see Supplementary Materials for Table S1). Many research studies did not discriminate between these two kinds of administration, since EOs were used to treat the food matrix on which pests moved and fed. However, we documented 74 papers claiming to evaluate contact toxicity, investigating the contact or the ingestion toxicity in 254 trials, each one involving a different combination of tested EO (or EO-based formulation) and target insect species. According to EO plant families, Lamiaceae (68 combinations), Asteraceae (33 combinations), Rutaceae (33 combinations), Psocoptera, Lepidoptera, Coleoptera, and other families were studied.
combinations), and Myrtaceae (20 combinations) were predominant. Most research studies focused on Coleoptera species (232 combinations), followed by Lepidoptera (13 combinations) and Psocoptera (10 combinations).

Regarding Coleoptera, many insect families were evaluated, although most efforts were directed toward Curculionidae (88 combinations) and Tenebrionidae (78 combinations). However, the effects of EOs on the mortality of stored product coleopteran species are highly variable. Abdelgaleil et al. [20] evaluated the toxic impact of 20 plant EOs against the curculionid S. oryzae, highlighting that only few plants exerted strong insecticidal contact activity. In detail, Artemisia judaica (Asteraceae), Callistemon viminalis (Myrtaceae), and Origanum vulgare (Lamiaceae) had LD50 values (i.e., the EO dose lethal for 50% of tested insects) of 0.08, 0.09, and 0.11 mg/cm², respectively. Promising results against S. oryzae were recorded also for EOs extracted from Syzygium aromaticum (Myrtaceae) and Lavandula officinalis (Lamiaceae) (LD50 values 0.04 and 0.07 mg/cm², respectively) [21], from Acorus calamus (Araeaceae) (LD50 value 54.46 μg/cm²) [22], and from Coriandrum sativum (Apiaceae), Eucalyptus obliqua L’Hér. (Myrtaceae), and Pinus longifolia (Pinaceae) (LD50 values 36.68, 52.77 and 77.30 μg/cm², respectively) [23]. However, the lowest LD50 values were recorded for Aster ageratoides (Asteraceae) (LD50 = 27.16 μg/cm²) [24], Dracophyllum moldavica (Lamiaceae) (LD50 = 22.10 μg/cm²) [25], and Litsea salicifolia (Lauraceae) (LD50 = 0.079 μL/insect) [26] against the close-related species Sitophilus zeamais (Motschulsky) (Coleoptera: Curculionidae), suggesting that these EOs may be reliable insecticidal sources also at very low dosages for curculionid stored product pests.

Overall, Tenebrionidae species were reported to be less susceptible to EOs compared with Curculionidae beetle [21, 22, 26]. Moreover, some plants showed important insecticidal activity against tenebrionid pests. In detail, Atalantia guillaumii (Rutaceae) presented an LD50 value of 17.11 μg/cm² [27] and Eucalyptus procer (Myrtaceae) an LD50 of 0.129 μL/cm² [28] against T. castaneum. For tenebrionid species, which are external feeders, also the ovicidal activity of EOs has been deemed. Also external pests (also known as secondary pests) develop for their whole life outside the grains, in contrast to internal feeders (or primary pests). Curculionid weevils are internal feeders, and their larval stages develop inside the kernels until the adult emergence, keeping them protected during the preimaginal stages.

Among Anobiidae species, research studies mainly involved Lasioderma serricorne F (Coleoptera: Anobiidae) and Callosobruchus spp. Among the tested EOs, L. serricorne showed higher susceptibility to Perilla frutescens EO (LD50 = 1.46 μg/adult) [29], while the bruchids Callosobruchus chinensis L. and Callosobruchus maculatus F. (Coleoptera: Bruchidae) were more susceptible to A. calamus (LD50 = 13.30 μg/cm²) and E. procer (LD50 = 0.124 μL/cm²), respectively [22, 28].

EO toxicity in Lepidoptera and Psocoptera was only evaluated toward Pyralidae and Liposcelididae species. E. kuehnii and Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae) are strongly susceptible to Satureja hortensis (Lamiaceae) (LD50 = 0.27 and 0.19 μL/cm², respectively) at the late larval stage [30]. The insecticidal activity of formulations against lepidopteran pests is usually evaluated toward the larvae, since the immature stages are responsible for direct food damage. Nevertheless, some studies also focused on the insecticidal toxicity of tested EOs toward adult moths, which are considered spreading agents and thus an important target for an appropriate pest-control program. However, contact toxicity against adult Lepidoptera is little investigated, since adult moths are generally less susceptible to EOs compared with Coleoptera species [23, 31]. Although this assumption is generally recognized, some EOs presented remarkable toxic activity against P. interpunctella adults; good mortality rates were reported for contact toxicity with Cymbopogon martini (Poaceae) EO (LD50 = 22.8 μg/cm²) [31], as well as for treatments with Coriandrum sativum (Apiaceae), which exerted an LD50 value of 47.95 μg/cm² [23]. Among Psocoptera, L. bostrychophila is the only psocid species studied for EO contact toxicity. Promising insecticidal activities were recorded for EOs extracted from Lagerra pterodonta (Asteraceae) (LD50 = 28.53 μg/adult) [32], Liriopse muscari (Asparagaceae) (LD50 = 21.37 μg/cm²) [33], and Dictannmus dasycarpos (Rutaceae) (LD50 = 27.2 μg/cm²) [34] used as contact insecticide.

When evaluating contact toxicity of EOs, it is not always possible to distinguish between the mere contact activity and the synergistic effect of ingestion and contact toxicity. For instance, several studies evaluate the toxicity by putting insect specimens on food grains treated with EOs. In this scenario, it is possible to hypothesize that EOs can act as contact insecticides, as well as they can exert ingestion toxicity when pests feed on the grains [35–40]. Few studies claimed to evaluate the ingestion toxicity of EOs. Popović et al. [41] evaluated the ingestion toxicity of 9 different EOs against T. castaneum, highlighting that at 1.14% EO concentration, only Calaminthia glandulosa (Req.) Benth. (Lamiaceae) showed good insecticidal outcome (i.e., over 96% mortality). In contrast, all the other tested EOs presented mortality rates lower than 15%. For instance, it is acknowledged that the presence of foodstuff, and thus the direct treatment of food, usually limits the toxicity of EOs toward stored product pests, suggesting that ingestion toxicity plays a minor role on pest mortality [42].

EOs are botanicals extracted from cultivated and wild plants, and their composition is strongly subjected to variations according to their geographic origin. Thus, it is not surprising that EOs from different geographic areas may cause different responses in the same insect species. As an example, Citrus sinensis L. (Rutaceae) EOs from geographically different origin presented highly variable LD50 value against S. oryzae, ranging between 0.29 and 0.43 mg/cm² [20, 21]. Furthermore, also the physiological status (i.e., flowering, vegetative, etc.) of the plants, as well as the part of the plant from which EOs were extracted, can significantly alter the quality and the quantity of plant-borne compounds and thus the toxicity of the EOs [43, 44].

The contact toxicity of EOs toward stored product pests may also be enhanced or reduced when EOs are combined...
with other control tools. A major criticism relative to EO employment as insecticide is their high volatility and thus low persistence. These characteristics force the operators to continuous and repeated applications. Several researches aimed at improving the stability of EOs through the combination with powders, which can be applied directly on foodstuffs. Indeed, some good results have been reported for montmorillonite clay, which could extend the effectiveness of Ocimum gratissimum (Lamiaceae) EO from 7 to 30 days against Sitophilus zeamais (Coleoptera: Curculionidae) [45]. Furthermore, also the employment of diatomaceous earths showed promising improvement of EO toxicity and consequently a strong reduction of employed EO dosages. Against curculionid and tenebrionid pests, the addition of diatomaceous earths may result in a 5- and 10-fold reduction, respectively, of the EO doses employed for the treatment [46]. On the contrary, Campolo et al. [47] demonstrated that diatomaceous earths could have antagonistic effect with C. sinensis EO in controlling Rhysopertha dominica (F.) (Coleoptera: Bostrichidae). In contrast, substituting diatomaceous earths with kaolin to treat wheat grain, EO-kaolin mixture exhibited synergistic toxic activity against the bostrichid pest. For instance, the particle size of diatomaceous earths, as well as of other clays and dusts, could strongly affect their effectiveness when combined with EOs. Ziaee et al. [48] investigated the combination of diatomaceous earths and Carum coticum (L.) (Apiaceae) EO toward T. confusum and Sitophilus granarius (L.) (Coleoptera: Curculionidae) adults, comparing specifically the particle sizes. This study revealed that while particles with dimensions >37 μm presented synergistic activity against both pests, bigger particles (>149 μm) had antagonistic effect toward T. confusum [48]. On this basis, the development of nanoformulations and nano-sized particles may be helpful to improve EO toxicity. Among these techniques, oil-loaded nanocapsules can improve EOs insecticidal activity, as reported for polycaprolactone nanocapsules loaded with Rosmarinus officinalis (Lamiaceae) EO against T. castaneum [49].

5. Fumigant Toxicity

According to the revised literature, 125 papers accounted the insecticidal activity of EOs through fumigation (see Supplementary Materials for Table S2), investigating 499 different trials, each one involving a different combination of tested EO (or EO-based formulation) and target insect species. The most studied families were Lamiaceae (167 combinations), followed by Asteraceae (56 combinations), Myrtaceae (49 combinations), Apiaceae (47 combinations), and Rutaceae (42 combinations). Similar to contact toxicity tests, the insect order most studied was Coleoptera (428 combinations), followed by Lepidoptera (68 combinations) and Psocoptera (4 combinations). Among Coleoptera, research studies mainly focused on Tenebrionidae (161 combinations), Curculionidae (139 combinations), and Bruchidae (49 combinations), while among Lepidoptera and Psocoptera, only Pyralidae (68 combinations) and Lepidoptera (428 combinations) were evaluated.

Some EOs showed to be highly effective against many Coleoptera species, according to their LC50 values (i.e., the EO concentration which caused 50% of mortality). Among Lamiaceae, Ocimum gratissimum-EO fumigation showed interesting insecticidal activity against R. dominica (LC50 = 0.20 μL/L), S. oryzae (LC50 = 0.50 μL/L), C. chinensis (LC50 = 0.20 μL/L), and Oryzaphilus surinamensis L. (Coleoptera: Silvanidae) (LC50 = 0.19 μL/L), although this EO was less active toward T. castaneum (LC50 = 24.9 μL/L) [50]. Similarly, fumigation with Artemisia scoparia-EO exerted LC50 values of 2.05 μL/L for T. castaneum, LC50 of 1.87 μL/L for S. oryzae and LC50 of 1.46 μL/L for C. maculatus [51].

Sitophilus oryzae is the most studied curculionid species and reveals to be particularly susceptible to C. coticum (Apiaceae) (LC50 = 0.91 μL/L) [52]. Lamiaceae were the most effective plant family. For instance, EOs from O. vulgare, Salvia fruticosa, S. officinalis, S. pomifera, Thymbra capitata, and Thymus perecutis showed high fumigation toxicity toward S. oryzae, with LC50 values ranging between 1.5 and 9 μL/L [20, 53, 54]. Among the other plant families, L. nobilis (Lauraceae) (LC50 = 8.0 μL/L) [53], Eucalyptus spp. (Myrtaceae) (LC50 = 7 and 8.5 μL/L) [55, 56], and Citrus limon (Rutaceae) (LC50 = 9.89 μL/L) [20] were particularly effective against S. oryzae adults applied as fumigant. Regarding other curculionid weevils, again Lamiaceae revealed to be very effective as fumigant (i.e., Origanum acutidens and Mentha pulegium against S. granarius [57, 58] and D. moldavica against S. zeamais [25]). Notably, also the EO from fruits of a plant belonging to the Lauraceae family, L. salicifolia, showed high insecticidal properties against S. zeamais when employed in fumigation trials (LC50 = 4.4 μL/L) [26]. Fumigation toxicity toward Curculionidae species mainly refers to adults. Indeed, as curculionid weevils are internal feeders, the toxicity of EOs toward larvae, pupae, and eggs has been little investigated and still inconclusive, although adults seemed to be more susceptible than the immature stages [59, 60].

Tribolium castaneum is an insect model to study fumigant toxicity of EOs. Among the reviewed papers, the most effective EO against T. castaneum was Allium sativum (Amaryllidaceae) with LC50 value of 1.52 μL/L [46]. As reported for Curculionidae, Lamiaceae EOs reveal strong toxicity also against T. castaneum. In detail, Rosmarinus officinalis (LC50 = 1.17 μg/mL) [61] and Mentha spp. (LC50 values between 12 and 13 μL/L after 24h) [62, 63] showed the highest insecticidal efficacy as fumigant agents. Furthermore, also the fumigations with EOs extracted from plants belonging to other plant families could have good knockdown abilities. Indeed, Achillea wilhelmsii (Asteraceae) gave good results against T. castaneum (LC50 = 10. 02 μL/L) [62], as well as Eucalyptus spp. (Myrtaceae) (LC50 values ranging between 11 and 14 μL/L) [28, 55, 56], Citrus reticulata (Rutaceae) (LC50 = 3.49 10-6%) [11] and Pistacia lentiscus (Anacardiaceae) (LC50 = 8.44 μL/L) [64].

Conversely to Tenebrionidae and Curculionidae, Asteraceae plants were generally more toxic against Bruchidae species [56, 62, 65, 66]. Nevertheless, the lowest recorded LC50 values were noted for Ocimum americanum, belonging to Lamiaceae, and Lippia multiflora, from
Verbenaceae, which were able to halve bruchid population at 0.23 and 0.47 μL/L, respectively [67]. Similar to Bruchidae, the EO from Artemisia herba-alba, (Asteraceae) was the most effective fumigant against O. surinamensis, with an LC50 value of 3.50 μL/L [68]. Furthermore, good knockdown outcomes were also obtained in fumigation trials applying EO from Myrtaceae toward R. dominica adults (Eucalyptus globules LC50 = 3.5 μL/L) as well as against L. serricorne adult insects treated with Lamiaceae EO (Lavandula stoechas LC50 = 3.8 μL/L) [69]. In contrast with previously described results, the bruchid Acanthoscelides obtectus (Say) (Coleoptera: Bruchidae) and the dermestid Trogoderma granarium (Everts) (Coleoptera: Dermestidae) seemed to be slightly influenced by EO fumigations [70–73]. Although the majority of EOs could exert good insecticidal activity against target insects as fumigants, some of them caused low or no toxicity against stored product pests if applied through fumigation [60, 70, 74, 75]. Nevertheless, research studies on the fumigant toxicity of EOs against adult insects of Coleoptera other than Curculionidae and Tenebrionidae were limited, and results may be less reliable and conclusive. Furthermore, it should be accounted that under real operative conditions, one of the factors which mainly affects EO-fumigation outcomes is the presence/absence of grain and food that can impair the effectiveness of the treatments [42].

As reported for contact toxicity, EO fumigant activity was just evaluated toward Pyralidae and Liposcelididae species among Lepidoptera and Psocoptera. EOs extracted was just evaluated toward Pyralidae and Liposcelididae adult insects of Coleoptera other than Curculionidae and Tenebrionidae species among Lepidoptera and Psocoptera. EOs extracted was just evaluated toward Pyralidae and Liposcelididae was just evaluated toward Pyralidae. Nevertheless, it has been claimed that tenebrionid eggs and pupae are generally less susceptible to EO fumigations than adults [60]. In contrast, among Lepidoptera, the toxicity of fumigated EOs has been recognized to be higher against larvae than adults and, among larvae, younger ones were more affected than older ones [83–85]. The ovicidal activity of EOs toward moth eggs was also investigated by Ayvaz et al. [86], reporting 100 % mortality for both E. kuehniella and P. interpunctella eggs when treated with S. thymbra (Lamiaceae) EO. Furthermore, this EO, when fumigated at the concentration of 50 μL/L, determined LT99 values (i.e., time occurring to have 99% of mortality) of 158.50 h and 81.88 h for the eggs of E. kuehniella and P. interpunctella, respectively [86]. Generally, higher concentration of EOs can reduce the lethal time and thus treatment duration [87].

As reported for contact toxicity, EO composition may vary according to its geographic origin, as well as to the plant part used for the extraction or to the extraction method, thus modifying its activity against stored product pests. Jemâa et al. [89] highlighted significant differences on T. castaneum and R. dominica mortality, attributable to geographic origin of L. nobilis leaves used for EOs extraction. Similarly, variable results may also be highlighted by research studies involving the same pest-plant but with different geographic origin [64, 89] or different EO extraction method [77, 90]. Furthermore, investigating the fumigant toxicity of EOs extracted from different plant parts has demonstrated that their toxicity may be deeply altered. As an example, the EOs from Cinnamomum camphora (Lauraceae) and Platycladi orientalis (Cupressaceae) fruits presented an insecticidal activity almost close to zero, compared with that recorded for EOs extracted from leaves and barks of the same plants [91, 92].

The synergistic effect of EOs with other compounds may enhance their fumigant toxicity. Thus, the formulation of EOs with other components, as well as the combination of EO fumigation with other treatments, may enhance plant-borne compounds insecticidal activity. Similar to contact toxicity trials, the combined effect of diatomaceous earths and fumigation with EOs was investigated, highlighting a synergistic effect of C. reticulata EO [93]. Remarkably, also the combination of gamma radiation and EOs was evaluated. Irradiation is used as a control tool against T. castaneum, but generally gamma radiations are used at high dosages to obtain good results. Thus, the combined effect of radiation and EOs may help reduce the doses of both “ingredients” by exploiting their synergistic effect. Ahmadi et al. [94] revealed that gamma radiations (230 Gy) may increase the insecticidal activity of R. officinalis and Perovskia atriplicifolia.
at 15.73 nL/cm² [98]. Tenebrionidae are the most studied after 12 days [96, 97]. Other nanoformulations as nanogels of myristic acid-controlled release of the active substances [49]. Furthermore, efficiency and persistence of EOs, guaranteeing a slow and release formulations. Polycaprolactone nanocapsules were routed to develop microencapsulation and other controlled release formulations. However, some of the major criticism of EO fumigations is the low persistence and the cost related to extended treatments. On this basis, many research efforts have been routed to develop microencapsulation and other controlled release formulations. Polyacaprolactone nanocapsules were proposed with good results in order to increase insecticidal efficiency and persistence of EOs, guaranteeing a slow and controlled release of the active substances [49]. Furthermore, other nanoformulations as nanogels of myristic acid-chitosan loaded by EOs were tested. Nanogels of C. copticum or C. cymimum revealed to be more toxic than the pure EOs, improving the persistence of C. copticum from 2–3 days to 21 days and maintaining C. cymimum toxicity around 60% after 12 days [96, 97].

6. Repellent Activity

Repellent activity of EOs toward stored product pests was investigated by 79 papers according to our research parameters (see Supplementary Materials for Table S3), 66 namely regarding repellence (224 pest-EO combinations), 8 feeding deterrence (41 combinations), and 5 oviposition deterrence (7 combinations). Combinations were defined as trials involving different combination of tested EO (or EO-based formulation) and target insect species. The plant families most evaluated were Lamiaceae (53 combinations), Rutaceae (44 combinations), Myrtaceae (28 combinations), Asteraceae (23 combinations), and Apiaceae (20 combinations), while the insect orders targeted were Coleoptera (221 combinations), Lepidoptera (30 combinations), and Psocoptera (21 combinations).

Among Coleoptera, the EOs from Zanthoxylum spp. (Rutaceae) were broad-spectrum repellents, since they were reported to repel both T. castaneum, and L. serricorne at 15.73 nL/cm² [98]. Tenebrionidae are the most studied Coleoptera family relative to repellence (77 combinations). Among the investigated EOs, Evodia spp. (Rutaceae) and P. frutescens (Lamiaceae) were repellent of class V (80.1–100% of repellency) at 7.86 nL/cm² after 4h [29, 99], while EOs from several Muraya spp. (Rutaceae), L. muscari (Asparagaceae), and Artemisia anethoides (Asteraceae) showed similar repellent results at higher concentration (15.73 nL/cm²) [33, 100, 101]. Apart from Tenebrionidae, Curculionidae (34 combinations) was also a widely studied insect family for EO repellence. In detail, S. zeamais was significantly repelled (class V) by Mentha longifolia subsp. capensis (Lamiaceae) and L. salicifolia (Lauraceae) [26, 102], while the closely related species S. oryzae was more repelled by Prangos acaulis (Apiaceae) [103]. Furthermore, when used to treat directly the food grain, O. gratissimum (Lamiaceae) EO was able to fully repel S. oryzae adults at 0.2 µL/g grain, as well as to completely deter the bruchid C. chinensis [50]. Among Bruchidae, treatments with the EOs from Chenopodium ambrosioides (Chenopodiaceae) and Adhatoda vasica (Lamiaceae) caused high repellent activity against both C. chinensis and C. maculatus [104]. However, EOs able to repel stored product pests at reasonable dosage and for prolonged times are very limited. As an example, the only EO able to highly repel Cryptolestes ferrugineus (Stephens) (Coleoptera: Cucujidae) adults was Citrus bergamia (Rutaceae), while EOs extracted from other Rutaceae, as well as from Lamiaceae species, were unable to cause significant repellence [105].

To assess the repellent activity of EOs toward stored product Coleoptera, Y-tube and wind tunnel have been also used [106–108]. Nevertheless, these approaches provided less reliable results, since they appeared to more properly evaluate the attractiveness of the tested compounds, as reported by Wang et al. [108]. However, these approaches may be helpful to highlight the behavioral responses of each single individual and to evaluate differences between sexes. As an example, in Y-tube trials, Pimienta-Ramirez et al. [109] demonstrated that S. zeamais females were repelled by Eupatorium glabratum (Asteraceae) EO, while the conspecific males were attracted by the same EO. Indeed, EOs are rich of plant secondary metabolites, some of which may be recognized by insects as food attractant or allomones.

Unfortunately, when testing the repellence activity, authors commonly did not calculate RD₅₀ values (i.e., the EO dose which determines 50% repellence of the tested insect), thus making comparisons among the outcomes of different EOs almost impossible. However, some interesting results have been reported for Pistacia lentiscus (Anacardiaceae) EO against several stored product pests, with low RD₅₀ value for all the tested insects. For instance, this EO showed RD₅₀ values of 0.015, 0.037, and 0.01 μL/cm² for T. confusum, S. zeamais, and R. dominica, respectively [110]. Nevertheless, regarding T. confusum, the most interesting result was obtained with M. pulegium (Lamiaceae) with RD₅₀ value of 0.025 [63]. In contrast, L. nobilis (Lauraceae) EO tested for repellence against T. confusum adults determined inconstant results (RD₅₀ = 0.045–0.139 μL/cm²), which were accountable to the geographic origin of the tested EOs [111]. Moreover, promising repellence of L. nobilis EO was documented toward R. dominica (RD₅₀ values ranging between 0.013 and 0.036 μL/cm² depending on the geographic origin) [111], while slight repellence was reported against L. serricorne adults (RD₅₀ = 37.84 μL/cm²) [112]. On the contrary, L. serricorne was more repelled by M. pulegium (Lamiaceae) and C. sativum (Apiaceae) EOs, noting RD₅₀ values of 0.01 and 0.049 μL/cm², respectively [63, 113]. Overall, less research studies addressed RD₅₀ of EOs against Curculionidae species. Furthermore, curculionid weevils are usually slightly less repelled than Tenebrionidae and Anobiidae species. Indeed, the best values of repellence for Sitophilus spp. were caused by EOs extracted from Cymbopogon spp.
(Poaceae) (RD$_{50}$ = 0.03 µL/cm$^2$) and C. sativum (Apiaceae) (RD$_{50}$ = 0.084 µL/cm$^2$) [113, 114].

Repellence activity of EOs toward Lepidoptera targeted mainly Pyralidae species (27 combinations). Furthermore, Allahvaii et al. [115] demonstrated that several EOs had similar repellency against the pyralid E. kuehniella and Sitotroga cerealella Olivier (Lepidoptera: Gelechiidae). Overall, among pyralid moths, E. kuehniella adults were generally less repelled by EOs than P. interpunctella ones [30, 116]. Indeed, the most effective EOs evaluated toward P. interpunctella were Anethum graveolens (Apiaceae) and R. officinalis (Lamiaceae), which presented 100% of repellency at the concentration of 2 µL/L of air [117], while regarding E. kuehniella, the highest repellence (84.2%) was recorded for L. nobilis (Lauraceae) at the same concentration [90].

Among Psocoptera, L. bostrychophila was the most studied species. This species was highly repelled (over 90%) by EOs extracted from L. muscari (Asparagaceae) and D. dasycarpus (Rutaceae) at 6.32 nL/cm$^2$, and good results (88% repellency) were also noted when psocids were exposed to A. guillauminii (Rutaceae) EO at 15.73 nL/cm$^2$ (V class repellent) [27, 33, 34].

Beside the evaluation of short-term persistence relative to the repellent ability of the EOs, it is essential to improve the effectiveness of this kind of treatment under operative conditions. However, few information is available on the persistence of EO repellence toward stored product pests. Nevertheless, the repellence of Cymbopogon nardus (Poaceae) EO toward T. castaneum was demonstrated to last at least 16 weeks, with repellency rates of 50% at 0.2 g/m$^2$ [118]. In this scenario, testing more stable and durable EO-based formulations may be helpful to increase the use of EOs as repellents under real conditions. Furthermore, formulation of EOs with other compounds, as well as formulations of different EOs, may enhance repellent ability by synergistic activity of the components. For instance, Ngassoum et al. [119] proved that Ocimum canum (Lamiaceae) could improve the repellence of Hyptis spicigera (Lamiaceae) EO against S. oryzae, conversely to Vepris heterophylla (Rutaceae) which caused an antagonistic effect.

The characteristic of EOs to act as antifeedant compounds is a key repellent mechanism, with a number of perspectives under operative conditions. Nevertheless, the repellence efficacy of EOs is usually reduced when they are applied directly on pest food. Furthermore, consumers may arise some concerns and perplexities about the quality and safety of food products after treatments with EOs. However, some research studies addressed this kind of repellent mechanism and tried to determine the effectiveness of food treatments. Promising feeding deterrence activity was recorded for Rutaceae and Chenopodiaceae EOs against bruchid beetles, with feeding deterrence index (FDI = % reduction feeding activity) reaching even 100% [104]. Good outcomes have been already demonstrated also for L. salicifolia (Lauraceae) against T. castaneum, for Eucalyptus floribunda (Myrtaceae) against R. dominica, and for Datura stramonium (Solanaeaceae) against C. ferrugineus [26, 120, 121]. On the contrary, the deterrent activity of EOs on feeding behavior is mainly species dependant. As an example, research studies, aimed at evaluating the antifeedant ability of EOs against T. granarium, highlighted that this pest was not particularly affected by EO administration, since the EOs of numerous different plant species did not alter significantly its feeding activity [121, 122]. As reported for repellence trials, the formulations of EOs with different materials could improve also their antifeedant efficacy. Thus, also the scale of the employed insecticide may influence its efficac. and the development of nanoparticle or nanoformulation can alter the antifeedant ability of plant-borne extracts. Werdin González et al. [39] demonstrated that, using polyethylene glycol (PEG) nanoparticles loaded with EOs, the nutritional physiology of both T. castaneum and R. dominica was specifically altered. While R. dominica adults were deterred more by the pure EOs than by PEG-EO nanoparticles, for T. confusum PEG formulation improved the antifeedant activity [39].

Another insectical mechanism which can be compared with repellence is oviposition deterrence. Although few studies addressed this interesting topic, remarkable oviposition deterrence was reported against E. kuehniella when the oviposition substrate was treated with Ziziphora clinopodioides (Lamiaceae) EO at 8000 ppm, reporting a reduction in the number of eggs laid of almost 90% [84]. In contrast, considering Coleoptera species, oviposition deterrence could usually appear at the highest tested dosages of EOs. Indeed, T. castaneum adult females were significantly repelled at the highest tested dose (70000 ppm) of Tagetes spp. (Asteraceae) EOs [65]. Similarly, the EO from Cinnamomum aromaticum (Lauraceae) showed good oviposition deterrence outcomes toward C. maculatus females at high dosages (62.85 µg/cm$^2$) [123].

7. Sublethal Physiological Effects

Many life-history traits of stored product pests may be slightly affected or deeply altered by EO treatments (see Supplementary Materials for Table S4). Indeed, plant-borne compounds may even not directly kill insects but could cause relevant reduction of the reproductive performances, as well as several developmental impairments. In the present review, we highlighted 20 papers addressing the impact of EOs on insect biological parameters. In detail, the majority of papers addressed the effect of EO-based treatment on the fecundity and the fertility, followed by research studies on other developmental parameters. As usual, most studies focused on Coleoptera species, while just few studies investigated the impact of EOs on Lepidoptera.

Insect reproductive ability mainly depends on fertility and fecundity of the populations. Here, the effects of EO-based treatments on (i) potential fecundity (i.e., number of eggs laid) (ii) fertility (i.e., the natural ability to produce offspring), and (iii) and lifetime fecundity (i.e., the actual reproductive rate) of stored product pests are reviewed.

When EOs were directly applied to foodstuffs, plant-borne compounds could act as oviposition deterrent, as well as ovi.cidal insecticides, reducing either the number of eggs laid and/or the percentage of hatched eggs. However, when the adult insects came in contact with and fed on EO-treated food, the presence of insecticidal molecules could also
influence the innate ability of female pests to lay viable eggs. As an example, Salvia and Eucalyptus spp. could strongly reduce, and even nullify, the number of eggs laid by bruchid females, with a reduction of oviposition inversely proportional to the increase in the EO dose employed [124, 125]. The reduction of potential fecundity in *C. chinensis* was not only related to the reduction of the egg-laying period (i.e., depending on the reduction of the lifespan of females), but it could also be attributed to disturbances during the vitellogenesis process. Interactions with the insect oocyte development were reported for the EOs extracted from *Artemisia herba-alba* (Asteraceae), *Salvia verbenaca* (Lamiaceae), and *Scilla maritima* (Amaryllidaceae), proving that EOs with high flavonoid content could significantly inhibit the egg-laying process and even the fertility of *C. chinensis* [124]. Apart from Bruchidae, different EOs were also able to reduce the lifetime fecundity and the number of eggs laid in *P. interpunctella*, when adult females were exposed to the botanicals for sublethal periods [31]. However, it is unclear if the potential fecundity reduction was attributable to a direct effect of the EOs on the gametogenesis or to an indirect disruption of the courtship and mating patterns of this species. Indeed, it has been demonstrated that residues of the EO could be adsorbed by *P. interpunctella* specimens and could modify male and female locomotion, thus restricting mating possibilities [31]. This kind of disruption of locomotion activity was also noted in some coleopteran species after EO application [48], impeding a clear understanding about the origins of fecundity reduction.

During fumigation with EOs, even short exposures to sublethal doses may affect pest fecundity and fertility. To determine the sublethal effects of the *Eucalyptus camaldulensis* (Myrtaceae) and *Heracleum persicum* (Apiaceae) EOs on *C. maculatus* females, a sublethal dose (i.e., LC$_{30}$) was tested for 24h as fumigation. After the treatment, the number of total and daily eggs laid for *C. maculatus* females was significantly reduced for both EOs, even if it was slightly higher (39.58% reduction) for *H. persicum* than for *E. camaldulensis* (27.58%) [126]. Fumigation with low doses of EOs could also alter fecundity in Lepidoptera. Adult females of the moth *P. interpunctella* were exposed to fumigation with LC$_{30}$ of various EOs for just 6h. Results revealed that *Artemisia khorasanica* (Asteraceae) and *Vitex pseudo-negundo* (Lamiaceae) EOs were able to reduce the potential fecundity of *P. interpunctella* by 17.71% and 12.11%, respectively. Similar to bruchid beetles, the reduction of potential fecundity was mainly attributable to the inferior daily egg production than a reduction in adult longevity [127]. Indeed, although adult moths presented shorter lifespans when exposed to EOs, egg laying was generally concentrated in the two days after mating, while later females produced few eggs per day [127]. Apart from potential fecundity, the exposure to *A. khorasanica* and *V. pseudo-negundo* EOs also reduced the fertility by 9.7% and 7.94%, respectively, as well as caused a decrease in larval weight [127]. For instance, fertility, and thus egg hatchability, could be prejudiced if the parental generation experienced EO treatments. As an example, egg viability could be impaired when adults come in contact with and/or feed on EO-treated grains or flour, as reported for *T. castaneum* [65]. In contrast to these results, poor effects were noted for the pyralid *E. kuehniella*, whose females after direct contact with *Ziziphora clinopodioides* (Lamiaceae) EO slightly modified their fecundity or fertility [84].

As previously reported, the longevity of pests after EO exposure may be significantly shortened, but it could not even strongly influence lifetime fecundity of stored product pests [126, 127]. Indeed, the literature reviewed here suggested that the reduction of lifetime fecundity was mainly attributable to a decrease in daily fecundity of insect females rather than to the reduction of female lifespan. Thus, the decrease in daily fecundity and in viability of laid eggs were the main factors related to the lower number of emerging adult offspring. Coleoptera species, as *T. castaneum* and *T. granarium*, showed great reductions of progeny production after parental exposure to the tested botanicals [72]. Particularly, *T. granarium* was more susceptible to plant products than *T. castaneum*. In detail, a complete reduction (100% inhibition) in F1 progeny of *T. granarium* was achieved with a concentration of 1.5% for the EOs of *Cinnamomum camphora* (Lauraceae) and *Ocimum basilicum* (Lamiaceae), while for *T. castaneum*, only the EO from *Pimpinella anisum* (Apiaceae) could completely nullify progeny production [72].

Residual insecticide activity of EOs could prevent adult emergence from pupae or impair the complete development of the larval stages [38]. Indeed, residues of EOs could remain in contact with pupae or larvae for a prolonged time when EOs are applied to the growing media, thus interfering with insect metabolism. For instance, Yang et al. [46] demonstrated that the reduction of progeny production of *S. oryzae* and *T. castaneum* after exposure to *Allium sativum* (Amaryllidaceae) EO was mainly attributable to the residual toxicity of the EOs on egg viability, as well as to its residual toxicity toward young larvae. Furthermore, when EO was used in combination with diatomaceous earths to prolong its persistence, F1 progeny was even greater inhibited [46]. Similar results on residual effect of EOs were reported for *R. dominica*, highlighting synergism between diatomaceous earths or kaolin with EOs. Since *R. dominica* females laid eggs over the grain surface and then larvae penetrate inside the kernels, the application of inert dusts increased progeny suppression, causing higher mortality rates at the stage of egg or young larva [47]. Lifetime progeny production could also be affected by the alteration and dilation of the developmental times (i.e., from egg to adult) of pests, which might consequently alter their doubling and generation times [127].

Essential oils are also known to act both as ingestion and antifeedant insecticides. The alteration of feeding activity might influence adult and larval performances, with particular reference to growth rate, food consumption, and food utilization. Nevertheless, Germinara et al. [42] suggested that the increased mortality of *S. granarius* adults exposed to sublethal concentrations of *L. angustifolia* (Lamiaceae) EO was not attributable to the ingestion toxicity, but to inhalation and contact toxicity of the plant-borne extract. Moreover, a direct effect of ingested EOs could not be
excluded for other insect species, since EO impact is species specific. Furthermore, reduction of insect-feeding activity could cause serious damage. Indeed, Eucalyptus floribundi (Myrtaceae) EO caused dose-dependent reduction of consumption rate toward both adult R. dominica and O. surinamensis and consequently caused a severe reduction of their growth rates [120].

8. Mode of Action

The intrinsic properties of EOs interfere with basic metabolic, biochemical, and physiological functions of insect pests (see Supplementary Materials for Table S5). In the lepidopteran species P. interpunctella, adults exposed to sublethal dosages of EOs extracted from Artemisia khorassanica (Asteraceae) and Vitex pseudo-negundo (Lamiaceae) produced larvae with significantly reduced energy content, by decreasing protein, lipid, and glycogen contents [127]. Thus, alterations attributable to EOs may be transferred by treated adults to the progeny. Furthermore, energy reservoirs are fundamental for lepidopteran attacking stored products, since as adults they generally limitedly feed or do not feed at all, exploiting the energy resources accumulated during preimaginal stages. Thus, a decrease of these kinds of resources at the larval stages may critically endanger insect survival and reproduction. Specifically, protein and lipid reserves are considered fundamental for reproductive parameters (i.e., egg production, fertility, and fecundity), while glycogen is generally linked to locomotion and flight ability.

Besides metabolic and physiological alteration, the ingestion of EOs may also produce histological modifications. Osman et al. [128] demonstrated that T. granarium larvae presented severe histological changes in their midguts concerning mainly the regenerative cells, thus causing the disruption of the epithelium and impairing the replacement of the functional epithelial cells. Moreover, the cells of hypodermis were necrotic and blackened, with no differentiation between exocuticle and endocuticle. Adults resulting from larvae treated with EOs presented fewer regenerative cells in the midguts, which were elongated with a narrower lumen and females presented germarium and follicular epithelium of the ovarioles with faint nuclei, prejudicing reproduction [128].

Several research studies showed neurotoxic actions of EOs, causing insect paralysis followed by death (reviewed by [129]). Among mechanisms of action, the inhibition of acetylcholinesterase (AChE) is one of the most investigated in stored product pests. AChE is one of the most important enzymes in neuronal and neuromuscular communication in insects and differs from mammalian enzyme by a single residue, making AChE an insect-selective target for newly developed insecticides. Essential oils were estimated to be a potential source of insecticides due to their ability to modifying the AChE activity of some stored product pests [20, 130, 131]. Studies on AChE-inhibitor activity of EOs were carried out on Coleoptera species, testing curculionid (8 insect-plant combinations) and bruchid (1 insect-plant combination) species. EOs from the following plants showed inhibition of AChE activity based on I$_{50}$ values (i.e., the concentrations of the tested essential oil that inhibited the in vitro-hydrolisis of substrate by 50%): Asteraceae (Artemisia judaica; Artemisia monosperma), Lamiaceae (Origanum vulgare), Myrtaceae (Callistemon viminalis; Melaleuca alternifolia), Rutaceae (Atalantia monophylla; Citrus aurantiifolia; Citrus limon). However, Abdelgaleil et al. [20] showed that S. oryzae adults may be differently affected by different EOs administration. Indeed, some EOs may present weak (A. monosperma: I$_{50}$ = 120 mg/L) or moderate (O. vulgare: I$_{50}$ = 61.3 mg/L) AChE inhibition, while EOs from other plants can cause significant inhibition. For instance, A. judaica showed the highest efficacy as AChE inhibitor (I$_{50}$ = 16.1 mg/L), followed by C. limon (I$_{50}$ = 20.2 mg/L), C. viminalis (I$_{50}$ = 28.5 mg/L), and C. aurantiifolia (I$_{50}$ = 29.4 mg/L).

Some EOs seem to be rather weak inhibitors of AChE, as also reported by Nattudurai et al. [131]. In this study, the effectiveness of Atalantia monophylla EO was evaluated against C. maculatus and S. oryzae, highlighting that insects of both species exposed to sublethal EO doses presented weak (i.e., less than 50% of inhibition at the highest tested dose) AChE-inhibitor responses. Indeed, AChE activity was decreased in the range of 10.96–45.21% at LC10 and LC30 doses in C. maculatus, as for S. oryzae a decrease of 9.18–44.90% was recorded at LC10 and LC30, respectively. However, A. monophylla EO affected the total esterase activity in insects since the authors registered a decrease of total esterases for both tested insects [131]. So far, esterases are known to be involved in the detoxification of foreign compounds and allelochemical volatiles. Similar to esterases, glutathione S-transferases (GSTs) are known to play a key role for insect detoxification mechanisms, with particular reference to their involvement in the neutralization and resistance mechanisms toward synthetic and natural insecticides [132, 133]. As already described for total esterase and AChE, the EO extracted from A. monophylla was also able to decrease GST activity. For instance, either C. maculatus or S. oryzae presented a reduction of GSTs of about 43% when the adult insects were treated with LC30 [131]. The ability of EOs to reduce and suppress the activity of detoxifying enzymes may improve the insecticidal efficacy of EO-based formulations, as well as be exploited as synergistic ingredient to enhance the efficacy of other insecticides.

In contrast to these results on C. maculatus and S. oryzae, Shojaei et al. [134] reported that the esterase activity in two Tenebrionidae species, T. castaneum and T. confusum, was not affected by the administration of Artemisia dracunculus (Asteraceae) EO, even at high dosages (LC70). Similarly, the production of mixed function oxidases (MFOs) was not significantly altered with respect to the untreated control, even at the highest EO dosage (LC70), in both T. castaneum and T. confusum. MFOs are considered as GSTs and esterases responsible of detoxifying ability in insects. On the contrary, species-specific responses were reported for GSTs. Treatment with EO slightly altered the GST production in T. confusum, by raising the GST activity according to concentration increase. Conversely, T. castaneum showed a decrease of GST production when EO concentration
increased. Nevertheless, control insects of both species showed the lowest enzyme activity, suggesting that EO administration enhanced the production of detoxifying enzymes as GST [134]. In this scenario, these results may shed light on the detoxification mechanism of some EO substances by tenebroid insects, but not the mode of action for this EO. Furthermore, even if no modification of esterase and MFO activity was clearly reported, the results might be impaired by the tested dosages chosen for these trials (LC30, LC50, and LC70), as lower concentrations could better detect alterations related to insect metabolism.

Metabolomic alterations caused by EO administration were also investigated for the curculionid S. zeamais using Melaleuca alternifolia (Myrtaceae) [130]. Indeed, M. alternifolia EO was shown to possess fumigant toxicity against S. zeamais along with the capacity to significantly inhibit the activity of 3 enzymes: two detoxifying enzymes, GST and carboxylesterase (CarE), as well as the nerve conduction enzyme AChE. In vivo enzyme inhibition was reported also for insects treated with EO dosages lower than LC50 (8.42, 7.70, and 6.78 mg/L air after 24, 48, and 72 h, respectively). For instance, M. alternifolia EO induced a moderate enzyme inhibition at the dose of 5.39 mg/L air after 12 h and 24 h for every tested enzyme (AChE, GST, and CarE), even if a certain restoration of enzyme activity could be noted after 24 h [130]. These results highlighted a pattern of significant dose- and time-dependent inhibitory effect of M. alternifolia EO on the enzyme activity in S. zeamais. The significant inhibition of the hydrolytic enzyme AChE caused by EO fumigation suggested that the EO might interfere with the nervous system of S. zeamais. Furthermore, since generally insects activate detoxifying enzymes to prevent and counterattack oxidative damage, the reduced activity of GST and CarE might improve the insecticidal activity of M. alternifolia EO.

To gain a better understanding of the mechanisms associated with the mode of action of EOs, Liao et al. [130] performed, for the first time, a comparative transcriptome analysis of S. zeamais in response to EO fumigation. The results from comparative transcriptome analysis on S. zeamais through RNA-Seq identified a total of 3,562 differentially expressed genes (DEGs), of which 2,836 and 726 were upregulated and downregulated, respectively, in response to M. alternifolia EO treatment. Interestingly, the majority of DEGs were involved in insecticide detoxification and mitochondrial function, followed by genes associated with respiration and metabolism of xenobiotics, including cytochrome P450s, CarEs, GSTs, and ATP-binding cassette transporters (ABC transporters). In detail, in the first phase of xenobiotic metabolism, which results in the alteration of xenobiotic compounds in more reactive molecules, CarEs and cytochrome P450 play an indispensable role. In S. zeamais, the transcription of genes encoding P450 was significantly upregulated, indicating that these genes might be involved in detoxification of M. alternifolia EO. CarEs unigenes were also upregulated upon oil exposure, in contrast to results from in vivo enzyme inhibition analyses after 12 h from exposure. In the second phase, the detoxifying enzymes further increase the water solubility of the metabolites, and GST is known to play an important role here. After exposure to M. alternifolia EO, S. zeamais adults presented 19 genes encoding GSTs upregulated, while 2 were downregulated, suggesting that insects try to recover from enzyme activity inhibition by increasing the production of different GSTs. Lastly, during the third phase, to transport conjugates of xenobiotic compounds out of the cell, ABC transporters were activated (30 genes upregulated). These results suggested the pathway used by S. zeamais to detoxify EO compounds [130].

However, over xenobiotic biodegradation, the alteration of mitochondrial functions, as the inhibition of respiratory enzymes or the alteration of regulation of oxygen/carbon dioxide ratio, may be a mode of action of plant EOs. Liao et al. [130] found that many genes associated with mitochondrial functions were differentially expressed, and some enzymes from the mitochondrial respiratory chain were downregulated by M. alternifolia EO treatment, causing the block of the electron flow by the hydrogen carrier and interfering with energy synthesis in the mitochondrial respiratory chain.

According to this hypothesis, adenosine triphosphatases (ATPases), a class of enzymes that catalyze the decomposition of ATP into ADP releasing energy, may be a target for EOs to impair chemical reactions, as well as respiration that would not otherwise occur. For the first time in insects, ATPases were found to be inhibited in S. oryzae adults exposed to different EOs (i.e., Artemisia judaica, Artemisia monosperma, Origanum vulgare, Callistemon viminalis, Melaleuca alternifolia, Atalantia monophylla, Citrus aurantifolia, and Citrus limon) [20]. In detail, the oils of C. viminalis, O. vulgare, and C. limon caused the highest enzyme inhibition with I50 values of 4.69, 6.07, and 9.69 mg/L, respectively, while EO from C. aurantifolia showed a slightly lower enzyme inhibition (I50 = 11.4 mg/L). In contrast, EOs from A. judaica (I50 = 21.4 mg/L) and A. monosperma (I50 = 24.6 mg/L) caused the weakest enzyme inhibition. Based on I50 values, in S. oryzae, EOs were more likely to inhibit the activity of ATPases than AChE one, suggesting that active compounds of EOs mainly affected energy chain reactions.

Overall, EOs are generally supposed to act as neuroinsecticides, and their insecticidal activity is considered species-dependent [129]. For this reason, in insects, other proposed mechanisms of EO action include the inhibition of GABA receptors (GABArs) and the alteration of the octopaminergic system. To the best of our knowledge, the ability of EOs to alter GABArs has never been proved for insects. On the contrary, modifications of the insect octopaminergic system following EO exposure have been already reported [135]. For instance, some EO components may compete with octopamine in binding to its receptor, causing an increase in the level of cAMP and calcium in nervous cells and modifying the neuron activity in Periplaneta americana L. (Blattodea: Blattidae) [135].

On this basis, it is possible to suggest that the broad-spectrum insecticidal activity of EOs could be attributable to the characteristics of these plant extracts, which are composed by numerous different compounds operating via several modes of action toward insect species.
9. Conclusions

Although an impressive increase in the number of publications involving botanical insecticides was recorded from 1980, as highlighted by Isman et al. [19], with over half of the papers on EOs (1,111) published in the last six years (2007/2012) of their survey period, the use of essential oils as insect-control tool in stored products still represents a niche compared with other sectors (i.e., crop protection, veterinary entomology, and mosquito control). Nevertheless, more than 200 papers have been published in the last 15 years. The increasing interest about essential oils derives from a number of factors such as their widespread availability, relatively low cost, and the belief that plant-borne extracts are nontoxic to humans and pets.

In the examined papers, EOs usually showed a noticeable acute toxicity (i.e., mortality), toward the target insects. This seems to be a foregone conclusion, given that these substances are synthesized by plants to defend themselves also from insects. Therefore, the question arises whether the feature is dose-dependent. And so it seems. Since many plants used for the EOs extraction often grow spontaneously in different natural habitats, their large-scale use should consider the cultivation of these essences to avoid negative impact in the ecosystems. Furthermore, many factors can influence the composition of essential oils. For example, the phenological stage and/or the part of the plant, the annual climatic variations, and the exposure can affect the relative amount of bioactive compounds constituting EOs. Thus, to validate the insecticidal activity of EOs and their potential as active ingredients for commercial pesticides, several trials should be carried out testing essential oils produced in different years and geographical areas.

Despite the promising results, there are few authorised commercial EO-based insecticide formulations available on the market. Future research studies about the mechanisms of action of the EOs against insects are needed to develop effective EO-based insecticides. Indeed, deeper knowledge on this topic may be helpful to estimate the impact of EOs toward nontarget species and their safety for consumers. In addition, the effect on the sensory analysis of food treated with these compounds should be evaluated since, although this aspect is a main concern for costumers, it has been often disregarded. Therefore, a multidisciplinary approach, involving also chemists and food technologists, could be a route to develop new EO-based insecticide formulations, which could be successfully applied to different productive sectors.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Supplementary Materials

The file contains 1 Supplementary Method and 5 Supplementary Tables: Supplementary Method 1: string used on Scopus database to retrieve the worldwide literature for scientometric analyses (Scopus database search: February 2, 2018). Table S1: overview of reviewed studies on EO contact (CT) and ingestion (IT) toxicity toward stored product pests. Table S2: overview of reviewed studies on EO fumigant toxicity toward stored product pests. Table S3: overview of reviewed studies on EO repellence toward stored product pests. Table S4: overview of reviewed studies on EO sublethal physiological effects toward stored product pests. Table S5: overview of reviewed studies on EO mode of action toward stored product pests. (Supplementary Materials)

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[69] A. Ebadollahi, M. H. Safaralizadeh, and A. A. Pourmirza, "Fumigant toxicity of essential oils of Eucalyptus globulus


