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Pesticidal Activity of Different Essential Oils

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CONTENTS

In the different chapters of Section II, "Essential and Vegetable Oils," a number of essential oils (EOs) are discussed in depth. Of course, the number of essential oils or plant species is much greater. In this chapter, first the pesticidal activities of some genera, *Lantana*, *Allium*, *Mentha*, and *Juniperus*, are detailed. Next, recent studies on essential oils of a wide range of plants are discussed. Finally, special attention is given to Chinese herbs.

23.1 *Lantana*

Dua et al. [1] investigated the insecticidal activity of essential oil isolated from the leaves of *Lantana camara* against mosquito vectors. The essential oil was isolated from the leaves of *L. camara* using the hydrodistillation method. A bioassay test was carried out by the World Health Organization's (WHO) method for determination of adulticidal activity against mosquitoes. LD_{50} values of the oil were 0.06, 0.05, 0.05, 0.05, and 0.06 mg/cm^2, while LD_{90} values were 0.10, 0.10, 0.09, 0.09, and 0.10 mg/cm^2 against *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles culicifacies*, *Anopheles fluvialitis*, and *Anopheles stephensi*, respectively. KDT_{50} values of the oil were 20, 18, 15, 12, and 14 min, and KDT_{90} values were 35, 28, 25, 18, and 23 min against *Ae. aegypti*, *C. quinquefasciatus*, *An. culicifacies*, *An. fluviatilis*, and *An. stephensi*, respectively, on 0.208 mg/cm^2 impregnated paper. Studies on the persistence of essential oil of *L. camara* on impregnated paper revealed that it has more adulticidal activity for longer periods at low storage temperature. Gas chromatography–mass spectrometry (GC-MS) analysis of the essential oil showed 45 peaks. Caryophyllene (16.37%), eucalyptol (10.75%), α-humelene (8.22%), and germacrene (7.41%) were present in major amounts and contributed 42.75% of the total constituents.

The biological properties of verbascoside are numerous and include antimicrobial activities. Leaf extracts of *Lippia javanica* Spreng. and *L. camara* Linné (Verbenaceae) were partially purified using column chromatography and high-speed centrifugal countercurrent

chromatography, the latter yielding fractions with higher purity (71%) than those from a single-column chromatographic separation (38%–44% pure) [2]. Verbascoside remained stable upon heating, but was completely decomposed after 4 h exposure of the extract to sunlight. Compared with the other storage conditions, the compound was best preserved in a dry form in the dark. Analysis by high-performance liquid chromatography revealed that the verbascoside content of plant parts of *L. camara* from natural populations was highly variable, both within and between populations. However, several specimens produced high levels of the compound (Hazyview, Plant 3 [83.0 mg/g dry weight], Magoebaskloof 2, Plant 5 [64.8 mg/g], and White River, Plant 2 [64.0 mg/g]), suggesting that *L. camara* is an excellent source of verbascoside. Extracts of the plant displayed effective *in vivo* inhibition of *Penicillium digitatum* on oranges.

Diaz Napal et al. [3] screened the activity of plant extracts derived from 89 species native to Argentina, against the leaf-cutting ant *Acromyrmex lundi* (Guérin) and its mutualistic fungus, *Leucoagaricus gongylophorus*, through a pickup assay and bioautography, respectively. The pickup assay revealed moderate to strong antiforaging activity for just over 13.5% of the assayed species, including complete ant foraging inhibition for *Aristolochia argentina*, *Flourensia oolepis*, *Gaillardia megapotamica*, *Lantana grisebachii*, and *Lithrea molleoides.* Most plant extracts were well tolerated by fungi, with only 12.3% of the species tested showing some degree of fungus growth inhibition. Among these, *A. argentina*, *F. oolepis*, and *Pterocaulon alopecuroides* were the strongest inhibitors, whereas *Baccharis flabellata*, *Dalea elegans*, and *Zanthoxylum coco* revealed a more moderate activity. Only *A. argentina* and *F. oolepis* extracts showed strong antiforaging effects and affected fungus growth at the same time. Values of IC_{50} and MIC indicated that extracts inhibiting ant foraging at lower concentrations did not necessarily also inhibit fungus growth at lower doses. The active principle of *A. argentina*, on both ant foraging and fungal growth, was identified as argentilactone.

Aromatic plants (i.e., *Citrus* spp., *Eucalyptus* spp., *L. camara*, *Ocimum* spp., and *L. javanica*) are commonly used in all the African studies of repellents against mosquito vectors [4]. Native people know three major methods of using repellent plants: (1) production of repellent smoke from burning plants, (2) hanging plants inside the house or sprinkling leaves on the floor, and (3) use of plant oils, juices from crushed fresh parts of the plants, or various prepared extracts applied on uncovered body parts.

The bioactivity of the essential oil extracted by hydrodistillation from *L. camara* leaves was assessed under laboratory conditions [5]. The composition of *L. camara* essential oil included large amounts of sesquiterpene, mainly β-caryophyllene (35.70%) and caryophyllene oxide (10.04%). The tested essential oil showed good fumigant activity within 1 week of exposure for all tested doses. Moreover, a remanence study confirmed that the oil was efficient during 2 weeks.

23.2 *Allium*

Junnila et al. [6] tested the efficacy of attractive toxic sugar bait (ATSB) with garlic oil microencapsulated in β-cyclodextrin as an active ingredient against *Aedes albopictus* in suburban Haifa, Israel. Two 3-acre gardens with high numbers of *Ae. albopictus* were selected for perimeter spray treatment with ATSB and ASB (bait containing no active ingredient). Baits were colored with food dye to verify feeding of the mosquitoes. The mosquito population was monitored by human landing catches and sweep net catches in the surrounding vegetation.

Experiments lasted for 44 days. Treatment occurred on day 13. The mosquito population collapsed about 4 days after treatment and continued to drop steadily for 27 days, until the end of the study. At the experimental site, the average pretreatment landing rate was 17.2 per 5 min. Two days posttreatment, the landing rate dropped to 11.4, and continued to drop to an average of 2.6 during the following 26 days. During the same period, the control population was stable. Few sugar-fed females (8%–10%) approached a human bait, and anthrone tests showed relatively small amounts of sugar within their crop or gut. Around 60%–70% of males caught near our human bait were sugar positive, which may indicate that the males were feeding on sugar for mating-related behavior. From the vegetation treated with the toxic bait, significantly fewer (about 10%–14%) males and females stained by ATSB than the ASB-treated control were recovered. This may indicate that the toxic baits alter the resting behavior of the poisoned mosquitoes within the vegetation. Almost no *Ae. albopictus* females (5.2 ± 1.4) approached human bait after treatment with ATSB.

Fresh garlic cloves were steam distilled to obtain the essential oil [7]. The garlic oil was tested for toxicity against the eggs, larvae, and adults of *Tribolium castaneum* and adults of *Sitophilus zeamais*. *T. castaneum* egg mortality increased with garlic oil concentration, with complete kill of eggs being achieved at 4.4 mg/cm^2, using the filter paper impregnation bioassay. The eggs were the most susceptible stage, followed by adults, 10-day-old larvae, and older larvae. *T. castaneum* adults were more susceptible to garlic oil than *S. zeamais* adults, with KD_{50} values of 1.32 and 7.65 mg/cm^2, respectively. When rice and wheat were treated with garlic oil, eggs that were laid in the media failed to produce F1 progeny at concentrations of 2000 ppm in rice for *T. castaneum* and 5000 ppm in wheat for *S. zeamais*. The weights of F1 adults of *T. castaneum* and *S. zeamais* in treated media were not significantly different from those of the controls.

In order to develop biological control of aphids by a "push–pull" approach, intercropping using repellent emitting plants was developed in different crop and associated plant models [8]. Garlic is one of the potential plants that could be inserted in crops to decrease the pest occurrence in neighboring crop plots. Field works were conducted in wheat fields in Langfang Experimental Station, Hebei Province, China, from October 2009 to July 2010 during the wheat developmental season. The effect of wheat intercropping with garlic, but also the volatiles emission on the incidence of the English grain aphid, *Sitobion avenae*, was assessed. Natural beneficial occurrence and global yields in two winter wheat varieties that were susceptible or resistant to cereal aphid were also determined, comparing with control plots without the use of garlic plant intercrop or semiochemical releaser in the fields. *S. avenae* was found to be lower in garlic oil blend (GOB) treatment, diallyl disulfide (DD) treatment, and wheat–garlic intercropping (WGI) treatment that compared the control plots for both varieties. Both intercropping and application of volatile chemicals emitted by garlic could improve the population densities of natural enemies of cereal aphid, including ladybeetles and mummified aphids. The ladybeetle population densities in WGI and GOB and the mummified aphid densities in WGI and DD were significantly higher than those in control fields for both varieties. There were significant interactions between cultivars and treatments to the population densities of *S. avenae*. The 1000-grain weight and yield of wheat were also increased compared with the control.

Water-distilled essential oil from the dried bulbs of *Allium chinense* (Liliaceae) was analyzed by GC-MS [9]. Eighteen compounds, accounting for 98.4% of the total oil, were identified, and the main components of the essential oil of *A. chinense* were methyl allyl trisulfide (30.7%), dimethyl trisulfide (24.1%), methyl propyl disulfide (12.8%), and dimethyl disulfide (9.6%), followed by methyl allyl disulfide (3.4%) and methyl propyl trisulfide (3.6%). The essential oil exhibited contact toxicity against the booklice (*Liposcelis bostrychophila*)

with a lethal concentration for 50% (LC_{50}) value of 441.8 μg/cm^2, while the two major constituents, dimethyl trisulfide and methyl propyl disulfide, had LC_{50} values of 153.0 and 738.0 μg/cm^2 against the booklice, respectively. The essential oil of *A. chinense* possessed strong fumigant toxicity against the booklice with an LC_{50} value of 186.5 μg/L, while methyl allyl trisulfide (LC_{50} = 90.4 μg/L) and dimethyl trisulfide (LC_{50} = 114.2 μg/L) exhibited stronger fumigant toxicity than methyl propyl disulfide (LC_{50} = 243.4 μg/L) and dimethyl disulfide (LC_{50} = 340.8 μg/L) against the booklice.

Dried bulbs of *A. sativum* were extracted with different solvents and evaluated for insecticidal, antimicrobial, and antioxidant activities.

Aqueous and methanol extracts showed the highest insecticidal activity (mortality rate of 81% and 64%, respectively) against the larvae of *Spodoptera litura* at a concentration of 1000 ppm [10]. With regard to antimicrobial activity, aqueous extract exhibited antibacterial activity against gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative (*Escherichia coli* and *Klebsiella pneumonia*) strains and antifungal activity against *Candida albicans*. While methanol extract showed antimicrobial activity against all the tested microorganisms except two (*S. aureus* and *C. albicans*), the extracts of hexane, chloroform, and ethyl acetate did not show any antimicrobial activity. The minimum inhibitory concentration of aqueous and methanol extracts against tested bacterial and fungal strains was 100–150 μg/ml. Antioxidant activity of the bulb extracts was evaluated in terms of inhibition of free radicals by 2,2′-diphenly-1-picrylhydrazyl. Aqueous and methanol extracts exhibited strong antioxidant activity (80%–90% of the standard).

Essential oil of *Allium macrostemon* was obtained by hydrodistillation and analyzed by gas chromatography (GC) and GC-MS [11]. The activities of the essential oil and its two major constituents were evaluated, using WHO procedures, against the fourth-instar larvae of *Ae. albopictus* for 24 h, and larval mortalities were recorded at various essential oil or compound concentrations ranging from 9.0 to 150 μg/ml.

The essential oil of *A. macrostemon* exhibited larvicidal activity against the early fourth-instar larvae of *Ae. albopictus* with an LC_{50} value of 72.86 μg/ml. The two constituent compounds, dimethyl trisulfide and methyl propyl disulfide, possessed strong larvicidal activity against the early fourth-instar larvae of *Ae. albopictus*, with LC_{50} values of 36.36 and 86.16 μg/ml, respectively.

23.3 *Mentha*

EO of Algerian *Mentha pulegium* L. leaves is obtained by steam distillation with a yield of 1.45% ± 0.01% and analyzed by GC-MS, where 39 compounds were identified [12]. The analysis has revealed that EO contains pulegone (70.66%) and neo-menthol (11.21%) as the major compounds. Antimicrobial study showed good activity tested against 11 bacteria (3 g+ and 8 g–) and 2 yeasts, and also showed good inhibitory (minimum inhibitory concentration [MIC]) and bactericidal (minimum bactericidal concentration [MBC]) properties.

Insecticide assessment was carried out against the food pest *Sitophilus granarius* (L.). Three toxicity tests of EO were performed using three different methods: contact, inhalation, and ingestion. Also, assessment of the toxicity of *M. pulegium* EO was carried out using leaves at different statuses (fresh, dried, and semidried), ground and nonground. Inhalation and ingestion methods were implemented on wheat seeds that showed high efficiency (100% mortality), and a lethal dose (LD_{50} = 9.11 ± 2.53 μl/ml) was obtained using

the contact method. Leaves in various statuses did not show any efficiency except crushed ones.

The essential oils of *M. pulegium* L. (MPE) and *Mentha rotundifolia* (L.) Huds (MRE), which are growing in Algeria, were prepared by hydrodistillation and their chemical compositions investigated by GC-MS [13]. The oils were tested for their antimicrobial activity using disc diffusion and spot assays, and antioxidant activity using the 2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) test and Kit Radicaux Libres® (KRL) biological assay. Also, contact toxicity, fumigant toxicity, and repellency tests of these essential oils were evaluated against adults of *Rhyzopertha dominica* (F.) (the principal pest of wheat). The major components found in MPE are pulegone (70.4%), neo-menthol (13.4%), neo-menthol acetate (3.5%), and menthone (2.7%). On the other hand, MRE provided *trans*-piperitone epoxide (30.2%), piperitone oxide (8.7%), thymol (4.5%), germacrene D (3.5%), and terpinen-4-ol (2.7%) as major ingredients. MRE exhibited a stronger antimicrobial effect and antioxidant activity in the KRL test than MPE. In the contact assay, DL_{50} values of MRE and MPE were 3.3 and 6.9 µl/ml, respectively. The fumigant toxicity assay of MPE and MRE showed mortality ratios of 39.2 and 44.3%, respectively, at the dose of 2 µl/ml. Moreover, at this dose and after 30 min exposure time, the repellent effect showed death rates of 46.03% and 47.54% for MPE and MRE, respectively.

See also Chapter 4 of this book.

23.4 *Juniperus*

The essential oil of *Juniperus procera* was evaluated against the larvae of *Anopheles arabiensis* under the laboratory and semifield conditions by adopting the World Health Organization standard protocols [14]. The larval mortality was observed for 24 h postexposure.

The essential oil of *J. procera* demonstrated varying degrees of larvicidal activity against *An. arabiensis*. The LC_{50} and LC_{90} values of *J. procera* were 14.42 and 24.65 mg/L, respectively, under laboratory conditions. However, under semifield conditions the LC_{50} and LC_{90} values of *J. procera* were 24.51 and 34.21 mg/L. The observations clearly showed that larval mortality rate is completely time and dose dependent compared with the control.

Sindhu et al. [15] report a new bioassay "syringe test" (modified larval immersion test [LIT]) for *in vitro* evaluation of acaricidal activity of crude plant extracts. Prepared syringes, containing eggs of tick, were incubated until 14 days after hatching of eggs, when the bioassay was performed on the larvae. LC_{50}, LC_{90}, and LC_{99} values were calculated for each tested product. Ninety-five percent confidence intervals for LC_{50} were very narrow, indicating a high degree of repeatability for the new bioassay on larvae of *Riphicephalus* (*Boophilus*) *microplus.* Bioassays were applied to six crude aqueous-methanol extracts from five plants (*Acacia nilotica*, *Buxus papillosa*, *Fumaria parviflora*, *Juniperus excelsa*, and *Operculina turpethum*), of which three showed discernible effects. Twenty-four hours postexposure, LC_{99} values were 11.9% (w/v) for *F. parviflora* and 20.8% (w/v) and 29.2% (w/v) for *B. papillosa* and *A. nilotica*, respectively. After six days of exposure, these values were 9.1% (w/v), 9.2% (w/v), and 15.5 (w/v) for *F. parviflora*, *A. nilotica*, and *B. papillosa*, respectively.

A laboratory-based study has been conducted to evaluate the repellency of the Ethiopian ethnomedicinal plant Tedh (vernacular name [local native language, Amharic], *J. procera* [Cupressaceae]) against the Afro-tropical malarial vector *A. arabiensis* Patton at four different concentrations: 1.0, 1.5, 2.5, and 5.0 mg/cm^2 [16]. Experimentation on the percentage of protection

in relation to the dosage has been performed. The tested concentrations of the essential oil of *J. procera* exhibited various degrees of repellency in terms of percentage of repellency and complete protection time against female *An. arabiensis*, that is, 1.0, 1.5, 2.5, and 5.0 mg/cm^2 (64.10% [92 min], 68.10% [125 min], 72.20% [190 min], and 80.60% [311 min], respectively). Student's *t*-test results showed a statistically significant difference between the treated and control groups.

The acaricidal effect of seven essential oils was examined *in vitro* against the cattle tick (*R. microplus*) [17]. Engorged female ticks were manually collected in farms of southern Brazil and placed into Petri dishes (n = 10) in order to test the following oils: juniper (*Juniperus communis*), palmarosa (*Cymbopogon martinii*), cedar (*Cedrus atlantica*), lemongrass (*Cymbopogon citratus*), ginger (*Zingiber officinale*), geranium (*Pelargonium graveolens*), and bergamot (*Citrus aurantium* var. *bergamia*) at concentrations of 1%, 5%, and 10% each. A control group was used to validate the tests containing Triton X-100 only. Treatment effectiveness was measured considering inhibition of tick oviposition (partial or total), egg's weight, and hatchability. *C. martinii*, *C. citratus*, and *C. atlantica* essential oils showed an efficacy higher than 99% at all concentrations tested. In addition, *J. communis*, *Z. officinale*, *P. graveolens*, and *C. aurantium* var *bergamia* oils showed efficiencies ranging from 73% to 95%, depending on the concentration tested, where higher concentrations showed greater efficacy.

23.5 Different EOs

The essential oils from accessions of *Lippia sidoides* (Verbenaceae) were characterized by GC and GC-MS and investigated for their acaricidal activity against the two-spotted spider mite (*Tetranychus urticae*) [18]. Twenty-nine compounds were identified with potential acaricidal activity. Glass receptacles were used as test chambers. For each dose and exposure time combination, three replicates were used. Each replicate consisted of 30 adult females of *T. urticae*, 10 mites in each leaf disc of *Canavalia ensiformis* placed in a Petri dish. Increasing amounts of oil or terpene were applied on a blotting paper strip, fixed on the inner surface of the glass recipient cover, corresponding to 2, 4, 6, 8, and 10 µl/L of air, respectively. Exposure periods were 24, 48, and 72 h. Data obtained in these experiments were submitted to probit analysis. The essential oil of *L. sidoides*, thymol, and carvacrol exhibited potent acaricidal activity against *T. urticae*.

Laboratory bioassays on insecticidal activity of essential oils extracted from six Mediterranean plants (*Achillea millefolium*, *Lavandula angustifolia*, *Helichrysum italicum*, *Foeniculum vulgare*, *Myrtus communis*, and *Rosmarinus officinalis*) were carried out against the larvae of the Culicidae mosquito *Ae. albopictus* [19]. The chemical composition of the six EOs was also investigated. All tested oils had insecticidal activity, with differences in mortality rates as a function of both oil and dosage. At the highest dosage (300 ppm), EOs from *H. italicum*, *A. millefolium*, and *F. vulgare* caused higher mortality than the other three oils, with mortality rates ranging from 98.3% to 100%. *M. communis* EO induced only 36.7% larval mortality at the highest dosage (300 ppm), a value similar to those recorded at the same dosage by using *R. officinalis* and *L. angustifolia* (51.7% and 55%, respectively). The analyzed EOs had a higher content of monoterpenoids (80%–99%) than sesquiterpenes (1%–15%), and they can be categorized into three groups on the basis of their composition. Few EOs showed the hydrocarbon sesquiterpenes, and these volatile compounds were generally predominant in comparison with the oxygenated forms, which were detected in lower quantities only in *H. italicum* (1.80%) and in *M. communis* (1%).

The efficacy of the essential oil and various organic extracts from flowers of *Cestrum nocturnum* L. was evaluated for controlling the growth of some important phytopathogenic fungi [20]. The oil (1000 ppm) and the organic extracts (1500 µg/disc) revealed antifungal effects against *Botrytis cinerea*, *Colletotrichum capsici*, *Fusarium oxysporum*, *Fusarium solani*, *Phytophthora capsici*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum* in the growth inhibition range of 59.2%–80.6% and 46.6%–78.9%, respectively, and their MIC values ranged from 62.5 to 500 µg/ml and 125 to 1000 µg/ml. The essential oil had a remarkable effect on spore germination of all the plant pathogens with concentration- and time-dependent kinetic inhibition of *P. capsici*. Further, the oil displayed remarkable *in vivo* antifungal effect up to 82.4%–100% disease suppression efficacy on greenhouse-grown pepper plants.

The acaricidal activity of a volatile essential oil hydrodistillate of *Satureja thymbra* L. (Lamiaceae) and its major constituents, carvacrol and γ-terpinene, was evaluated against field-collected unfed adult *Hyalomma marginatum* [21]. The distillate was tested against this tick species at 5, 10, 20, and 40 µl/L, while the two major components were each tested at 10 µl/L. Generally, tick mortality to the *S. thymbra* distillate increased with concentration and exposure time. Ticks exposed to vapors from cotton wicks containing at least 40 µl/L resulted in complete (100%) mortality at 3 h. The lower concentrations provided ≥90% mortality at 3 h posttreatment, with complete mortality at 24 h. Knockdown was observed only in the carvacrol and γ-terpinene treatments. Ticks exposed to carvacrol-treated wicks produced >93% knockdown at 3 h, but at 24 h, approximately 57% were dead. The γ-terpinene treatment produced ≥90% knockdown at 105 min through 3 h, but at 24 h, only about 87% of the ticks were dead.

Prakash et al. [22] report the essential oil of *Ocimum gratissimum* as a plant-based preservative and recommend its application as a nontoxic antimicrobial and antiaflatoxigenic agent against fungal and aflatoxin contamination of spices, as well as a shelf-life enhancer in view of its antioxidant activity. The EO exhibited antifungal activity against fungal isolates from some spices and showed better efficacy as a fungitoxicant than the prevalent fungicide Wettasul-80. The EO showed antioxidant activity through 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and β-carotene–linoleic acid bleaching assay. Methyl cinnamate (48.29%) and γ-terpinene (26.08%) were recorded as the major components of the oil through GC-MS analysis. The EO was found to be nonmammalian toxic, showing a high LD_{50} (11622.67 µl/kg) during oral toxicity on mice.

The bioactivity of the essential oil extracted by hydrodistillation from the seed of *Coriandrum sativum* was assessed under laboratory conditions for its biological activity against *S. granarius* in chickpea grains [23]. The components of the essential oil were identified through GC and GC-MS. The identity of 17 constituent compounds of the essential oil was confirmed, and their relative proportion was determined. Linalool has the highest percentage composition in the *C. sativum* seed essential oil (73.11%). All essential oil dosages showed a significant level of toxicity to the insect after 5 days.

Hesperozygis ringens (Lamiaceae) is a strongly aromatic plant employed popularly for its antiparasitic properties [24]. The leaves afforded 4% essential oil, constituted mainly by pulegone (86%). Laboratory tests were carried out to determine the toxicity of the essential oil species on engorged females and larvae of the cattle tick *R. microplus* using the adult immersion test (AIT) and the LIT. It was observed that the essential oil at the concentration of 50 and 25 µl/ml inhibited the egg laying significatively in relation to the controls, and the eggs from these treated females were affected by the oil; the hatching was inhibited in 95% and 30%, respectively. In the LIT, it was verified that the $LC_{99.9}$, LC_{50}, and LC_1 were 0.541, 0.260, and 0.015 µl/ml, respectively. Pulegone, isolated from the oil, showed a similar effect on the females and the larvae, indicating that it is responsible for the oil activity.

In order to identify natural products for plant disease control, the essential oil of star anise (*Illicium verum* Hook. f.) fruit was investigated for its antifungal activity on plant pathogenic fungi [25]. The fruit essential oil obtained by hydrodistillation was analyzed for its chemical composition by GC and GC-MS. *trans*-Anethole (89.5%), 2-(1-cyclopentenyl)-furan (0.9%), and *cis*-anethole (0.7%) were found to be the main components among 22 identified compounds, which accounted for 94.6% of the total oil. Both the essential oil and *trans*-anethole exhibited a strong inhibitory effect against all test fungi, indicating that most of the observed antifungal properties were due to the presence of *trans*-anethole in the oil, which could be developed as a natural fungicide for plant disease control in fruit and vegetable preservation.

Essential oil of *Plectranthus amboinicus* was studied for its chemical composition and larvicidal potential against the malarial vector mosquito *An. stephensi* [26]. In total, 26 compounds were identified by GC and GC-MS. The major chemical compound was carvacrol (28.65%), followed by thymol (21.66%), α-humulene (9.67%), undecanal (8.29%), γ-terpinene (7.76%), ρ-cymene (6.46%), caryophyllene oxide (5.85%), α-terpineol (3.28%), and β-selinene (2.01%). The larvicidal assay was conducted to record the LC_{50} and LC_{90} values, and the larval mortality was observed after 12 and 24 h of exposure. The LC_{50} values of the oil were 33.54 ppm (after 12 h) and 28.37 ppm (after 24 h). The LC_{90} values of the oil were 70.27 ppm (after 12 h) and 59.38 ppm (after 24 h). The results of the present study showed that the essential oil of *P. amboinicus* is one of the inexpensive and eco-friendly sources of natural mosquito larvicidal agent to control or reduce the population of malarial vector mosquitoes.

The aim of the research of Liu et al. [27] was to determine larvicidal activity of the essential oil derived from roots of *Saussurea lappa* (Compositae) and the isolated constituents against the larvae of the Culicidae mosquito *Ae. albopictus*. The essential oil of *S. lappa* roots was obtained by hydrodistillation and analyzed by GC and GC-MS. A total of 39 components of the essential oil of *S. lappa* roots were identified. The essential oil has a higher content (79.80%) of sesquiterpenoids than monoterpenoids (13.25%). The principal compounds in *S. lappa* essential oil were dehydrocostus lactone (46.75%), costunolide (9.26%), 8-cedren-13-ol (5.06%), and α-curcumene (4.33%). Dehydrocostus lactone and costunolide exhibited strong larvicidal activity against *Ae. albopictus* with LC_{50} values of 2.34 and 3.26 μg/ml, respectively, while the essential oil had an LC_{50} value of 12.41 μg/ml.

The objectives of the study of Tian et al. [28] were to determine the antifungal activity *in vitro* of the essential oil extracted from the seeds of dill (*Anethum graveolens* L.) and to evaluate its antifungal activity *in vivo* as a potential food preservative. The antifungal activity of this oil was tested by a poisoned food technique against *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus niger*, and *Alternaria alternata*. The wet and dry mycelium weights of the tested fungi were also determined in a liquid culture to evaluate the antifungal activity. The minimum inhibitory concentration of oil for the four tested fungi was found to be 2.0 μl/ml, and the mycelial growth inhibition was determined at day 9. The effect of the essential oil on inhibition of decay development on cherry tomatoes was tested *in vivo* by exposing inoculated and control fruit to essential oil vapor at 120 and 100 μl/ml concentrations, respectively.

The essential oil composition and *in vitro* antioxidant and antimicrobial activity of the essential oil and methanol extract of *Salvia eremophila* were evaluated in the research of Ebrahimabadi et al. [29]. GC and GC-MS analysis of the plant essential oil resulted in the identification of 28 compounds representing 99.24% of the oil. Borneol (21.83%), α-pinene (18.80%), bornyl acetate (18.68%), and camphene (6.54%) were detected as the major components, consisting of 65.85% of the oil. The plant essential oil and methanol

extract were also subjected to screenings for the evaluation of their antioxidant activities using DPPH and β-carotene–linoleic acid tests. While the plant essential oil showed only weak antioxidant activities, its methanol extract was considerably active in both DPPH (IC_{50} = 35.19 μg/ml) and β-carotene–linoleic acid (inhibition percentage 72.42%) tests. The plant was also screened for its antimicrobial activity, and good to moderate inhibitions were recorded for its essential oil and methanol extract against most of the tested microorganisms.

The aspects of the antifungal activity of essential oil of laurel (*Laurus nobilis*) obtained by means of a supercritical carbon dioxide (SFE-CO_2) technique against postharvest spoilage fungi have been studied by tests performed under *in vitro* and *in vivo* conditions [30]. The determination of the main active substances was carried out by gas chromatography analysis: laurel oil was characterized by a high content (≥10%) of 1,8-cineole, linalool, terpineol acetate, and methyl eugenol and a low content (<10%) of linalyl acetate, eugenol, sabinene, β-pinene, and α-terpineol. The inhibition of the mycelial growth of *B. cinerea*, *Monilinia laxa*, and *P. digitatum* was evaluated *in vitro* at concentrations of 200, 400, 600, 800, and 1000 μg/ml. *M. laxa* was totally inhibited by application of the oil at the lowest concentration, *B. cinerea* was completely inhibited at the highest concentration, and a fungistatic action was observed in both cases. *P. digitatum* was only partially inhibited at all the concentrations. The activity of the oil, placed in the form of spray on the fruit skin at concentrations of 1, 2, and 3 mg/ml, was studied by biological tests. Both curative and protective activities of the oil have been evaluated on peaches, kiwifruits, oranges, and lemons artificially inoculated with *M. laxa*, *B. cinerea*, and *P. digitatum*, respectively. A very good antifungal activity was found on kiwifruits and peaches when the oil was placed before the inoculation at a concentration of 3 mg/ml (68% and 91% of decay inhibition, respectively). The same activity was found on peaches when the oil was placed after the infection (76% of decay inhibition). The application of the oil did not cause any phytotoxic effect and kept any fruit flavor, fragrance, or taste.

Mishra et al. [31] report on fungal deterioration of five herbal raw materials and the antifungal, antiaflatoxigenic, and antioxidant efficacy of Jamrosa essential oil and its two major components. Herbal raw materials were found to be associated with 14 fungal species, including strains of aflatoxin-producing *A. flavus.* Jamrosa EO and its major components Z-citral and linalyl acetate were assessed against the highest aflatoxin B_1 (AFB_1)–producing strain, *A. flavus* $LHPA_9$. The Jamrosa EO MIC for *A. flavus* $LHPA_9$, as well as the concentration that suppressed aflatoxin B_1 production, was 0.4 μl/ml. This EO was found to be more efficacious than its major components individually, as well as in combination. Z-citral inhibited AFB_1 completely at 1.0 μl/ml, while linalyl acetate did so at 0.7 μl/ml. The combination of both compounds completely inhibited AFB_1 production at 0.8 μl/ml. Free radical scavenging activities (IC_{50}) of EO, Z-citral, linalyl acetate, and a combination of both compounds were 86, 94, 217, and 158 μl/ml, respectively.

The essential oil of *Deverra scoparia* Coss. & Durieu was investigated for its acaricidal activity against a worldwide pest, the two-spotted spider mite, *T. urticae* Koch (Acari: Tetranychidae) [32]. The essential oil was analyzed by fast GC and GC-MS. The activities of its individual and blended constituents were determined. This study showed that female mortality increased with increasing *D. scoparia* oil concentrations, with LD_{50} and LD_{90} values at 1.79 and 3.2 mg/L, respectively. A reduction in fecundity had already been observed for concentrations of 0.064, 0.08, and 0.26 mg/L *D. scoparia* essential oil. Ten major components, comprising 98.52% of the total weight, were identified; α-pinene was the most abundant constituent (31.95%), followed by sabinene (17.24%) and Δ3-carene (16.85%). The 10 major constituents of *D. scoparia* oil were individually tested against *T. urticae* females.

The most potent toxicity was found with α-pinene, Δ3-carene, and terpinen-4-ol. The presence of all constituents together in the artificial mixture caused a significant decrease in the number of eggs laid by females, at 0.26 mg/L (11 eggs), compared with the control (50 eggs). The toxicity of blends of selected constituents indicated that the presence of all constituents was necessary to reproduce the toxicity level of the natural oil.

Essential oil of *Satureja hortensis* isolated via hydrodistillation was investigated against 1- to 7-day-old adults of the red flour beetle, *T. castaneum* (Herbst); 12- to 14-day-old larvae of the Mediterranean flour moth, *Ephestia kuehniella* (Zell.); and Indian meal moth, *Plodia interpunctella* (Hübner) [33]. Repellency of this oil on all three pest species' adults was also studied. After 48 h of exposure, the LC_{50} value for *T. castaneum* was 192.35 μl/L. LC_{50} values were calculated as 80.9 μl/L and 139.8 μl/L after 9 h for *E. kuhniella* and *P. interpunctella*, respectively. *S. hortensis* oil showed more contact toxicity against *P. interpunctella* (LC_{50} = 0.19 μl/cm^2) than *E. kuehniella* (LC_{50} = 0.27 μl/cm^2). Repellency of this oil on all the insect species was high. The relationship between exposure time and oil concentration on the mortality of all species indicated that mortality was increased by increasing the oil concentration and exposure time.

The essential oil from *Hyptis suaveolens* L. (Lamiaceae) was analyzed by GC and gas chromatography–electron impact mass spectroscopy (GC-EIMS), and 66 constituents were identified [34]. *H. suaveolens* EO contains sabinene (34%), β-caryophyllene (11.2%), terpinolene (10.7%), β-pinene (8.2%), limonene (5.8%), and 4-terpineol (2.5%) as major constituents. Moreover, *H. suaveolens* EO and its major constituents were evaluated for their repellent activity against adults of the granary weevil *S. granarius* (L.) (Coleoptera: Dryophthoridae) in Petri dish tests and in pitfall bioassays. Data showed that *H. suaveolens* EO possesses a noticeable repellent activity against *S. granarius* in both testing methods. Furthermore, in all trials good repellence rates of terpinolene, β-pinene, and sabinene were found, in particular at lower dosages.

Zapata and Smagghe [35] report on the repellent activity, as well as contact and fumigant toxicity, of four essential oils extracted from the leaves and bark of *Laurelia sempervirens* and *Drimys winteri* against an important stored product insect pest: the red flour beetle, *T. castaneum*. The four oils tested had a very strong repellent activity toward *T. castaneum* when tested in a filter paper arena test. After 4 h exposure, >90% repellency was achieved with *L. sempervirens* oils at low concentrations of 0.032 μl/cm^2, while for *D. winteri* oils, concentrations of 3–10 times higher were needed to achieve this activity. Oils of both *L. sempervirens* and *D. winteri* were found to be toxic toward *T. castaneum* when applied topically or by fumigation. LD_{50} values by topical application of *L. sempervirens* oils were from 39 to 44 μg/mg of insect; for *D. winteri* oils, these were from 75 to 85 μg/mg of insect. By fumigation, LC_{50} values for *L. sempervirens* oils were 1.6–1.7 μl/L air, while these were 9.0–10.5 μl/L air for *D. winteri* oils. In addition, with *L. sempervirens* oils, 50% of the tested beetles were killed at 100 μl/L air within 3.0–4.4 h, while with *D. winteri* oils, the LT_{50} values were 6.1–7.4 h.

Oregano essential oil (*Origanum onites*) was applied at two doses, 0.55 and 0.75 μl/cm^3, and two exposure times, 3 and 6 h, as a disinfectant for hatching eggs [36]. The formaldehyde-treated eggs were used as a positive control, and untreated eggs were used as a negative control. After chemical analysis, the main constituents of oregano essential oil were carvacrol, linalool, para-cymene, and γ-terpinene. The lowest microbial counts on eggs were obtained from oregano essential oil. Microbial inhibition increased with the increasing essential oil concentrations. Essential oil exposure times had no significant effects on microbial counts. Essential oil fumigation lowered middle embryonic mortality and the discarded chick rate, but increased early and late embryonic mortalities compared with formaldehyde treatment. Essential oil doses significantly affected late embryonic

mortality, the discarded chick rate, the contamination rate, hatchability of the fertile egg, the body weight at 21 and 42 days, body weight gain, and total feed consumption. But, early and middle embryonic mortality were not significantly affected by treatments.

The cowpea weevil, *Callosobruchus maculatus* (F.), is a major pest of cowpea *Vigna unguiculata* (L.) Walp. in storage units, making the grains unsuitable for consumption [37]. The aim of this study was to obtain and chemically identify the components of essential oils extracted from fruit peels of *Citrus latifolia* Tanaka, *Citrus reticulata* Blanco, *Citrus sinensis* L. Osbeck, and *Citrus paradisi* Macf., as well as to determine the contact and fumigant toxicity of these oils and their repellent effect on *C. maculatus* adults. GC-MS analysis identified 45 compounds in the essential oils; the major components were described as follows: *C. latifolia* (limonene 57.7%, γ-terpinene 17.2%, β-pinene 12.3%, and α-pinene 2.0%), *C. sinensis* (limonene 93.8% and myrcene 2.1%), *C. reticulata* (limonene 94.2% and myrcene 1.6%), and *C. paradisi* (limonene 94.2% and myrcene 1.8%). In the contact toxicity tests using treated cowpeas, the LC_{50} values ranged from 943.9 to 1037.7 ppm, with the lowest value for *C. latifolia* and the highest for *C. sinensis*. The number of eggs and newly emerged adults was inversely proportional to the essential oil concentration increase. In the fumigant toxicity test, LC_{50} values ranged from 10.2 to 12.98 µl/L air, with *C. latifolia* showing the best results. In the repellency test, the essential oils were classified as neutral at all concentrations. The percentages of oviposition reduction ranged from 29.74% to 71.66%, while reduction in emergence varied from 15.43 to 85.31.

The purpose of the review of Pavela [38] was to evaluate the current research on using EOs as potential larvicides based on their chemical composition and biological efficacy. The selected plants (their EOs), as the case may be, were therefore required to meet two essential conditions: (1) $LC_{50} \leq 100$ ppm and (2) their chemical composition had to be known.

In total, 122 plant species from 26 families were selected from the available literature. However, more than two-thirds of the plants (68.8%) were from only five families: Lamiaceae, Cupressaceae, Rutaceae, Apiaceae, and Myrtaceae.

Considering the above-estimated LC_{50} value as the main criterion of efficacy, 77 showed $LC_{50} < 50$ ppm. Some of these efficient EOs were obtained from aromatic plants also grown commercially on relatively large areas, with good cultivation technology (e.g., *Pimpinella anisum*, *C. sativum*, *F. vulgare*, *Mentha longifolia*, *Ocimum basilicum*, *Thymus* spp., *Eucalyptus* spp., and *Piper* spp.). Such plants could become a suitable source of active substances for potential botanical larvicides. Only seven plants (*Blumea densiflora*, *Auxemma glazioviana*, *Callitris glaucophylla*, *Cinnamomum microphyllum*, *Cinnamomum mollissimum*, *Cinnamomum rhyncophyllum*, and *Zanthoxylum oxyphyllum*) can be considered significantly most efficient, given that $LC_{50} < 10$ ppm has been estimated for their EOs. These EOs contained less common substances, predominantly from the group of sesquiterpenes, aromatic acids, and ketones.

23.6 Chinese Herbs

In the screening program for new agrochemicals from Chinese medicinal herbs, *Murraya exotica* was found to possess insecticidal activity against the maize weevil, *S. zeamais*, and red flour beetle, *T. castaneum* [39]. The essential oil of aerial parts of *M. exotica* was obtained by hydrodistillation and investigated by GC and GC-MS. The main components of *M. exotica* essential oil were spathulenol (17.7%), α-pinene (13.3%), caryophyllene oxide

(8.6%), and α-caryophyllene (7.3%). Essential oil of *M. exotica* possessed fumigant toxicity against *S. zeamais* and *T. castaneum* adults with LC_{50} values of 8.29 and 6.84 mg/L, respectively. The essential oils also show contact toxicity against *S. zeamais* and *T. castaneum* adults with LD_{50} values of 11.41 and 20.94 mg/adult, respectively.

In the same screening program for new agrochemicals from Chinese medicinal herbs and wild plants, essential oil of *Kadsura heteroclite* stems was found to possess strong toxicities against the root-knot nematode, *Meloidogyne incognita*, and the maize weevil, *S. zeamais* [40]. The essential oil of *K. heteroclita* was extracted via hydrodistillation and analyzed by gas chromatography–flame ionization detector (GC-FID) and GC-MS. A total of 46 components of the essential oil were identified. The main components of the essential oil were α-eudesmol (17.56%), 4-terpineol (9.74%), δ-cadinene (9.27%), and δ-cadinol (6.32%), followed by δ-4-carene (4.78%) and calarene (4.01%). The essential oil exhibited strong nematicidal activity against *M. incognita*, with an LC_{50} value of 122.94 μg/ml. The essential oil possessed contact toxicity against *S. zeamais* adults, with an LD_{50} value of 25.57 μg/adult, and also showed pronounced fumigant toxicity against *S. zeamais* (LC_{50} = 14.04 mg/L air).

This screening for bioactive principles from several Chinese medicinal herbs showed that the essential oil of *Cymbopogon distans* aerial parts possessed strong repellency against the booklouse, *L. bostrychophila*, and the red flour beetle, *T. castaneum* [41]. A total of 36 components of the essential oil were identified by GC and GC-MS. *trans*-Geraniol (16.54%), (*R*)-citronellal (15.44%), (+)-citronellol (11.51%), and α-elemol (9.06%) were the main components of the essential oil, followed by β-eudesmol (5.71%) and (+)-limonene (5.05%). Geraniol and citronellol were strongly repellent against *L. bostrychophila*, whereas citronellal and limonene exhibited weak repellency against the booklouse. Geraniol and citronellol exhibited comparable repellency against the booklouse relative to the positive control, DEET. Moreover, geraniol and citronellol exhibited stronger repellency against the red flour beetle than DEET, whereas the two other compounds showed the same level of repellency against the red flour beetle compared with DEET.

In the screening program of Chu et al. [42] for new agrochemicals from local wild plants, essential oil of *Artemisia vestita* (Asteraceae) was found to possess strong insecticidal activity against the maize weevil, *S. zeamais*. Essential oil of aerial parts of *A. vestita* was obtained from hydrodistillation and was investigated by GC and GC-MS. The main components of essential oil were grandisol (40.29%), 1,8-cineol (14.88%), and camphor (11.37%). The essential oil of *A. vestita* possessed strong fumigant toxicity against *S. zeamais* adults, with an LC_{50} value of 13.42 mg/L air. The essential oil of *A. vestita* also showed contact toxicity against *S. zeamais* adults with an LD_{50} value of 50.62 mg/adult.

The essential oil of *Rhododendron anthopogonoides* flowering aerial parts possesses significant toxicity against maize weevils, *S. zeamais* [43]. A total of 37 components were identified in the essential oil, and the main constituents of the essential oil were 4-phenyl-2-butanone (27.22%), nerolidol (8.08%), 1,4-cineole (7.85%), caryophyllene (7.63%), and γ-elemene (6.10%), followed by α-farnesene (4.40%) and spathulenol (4.19%). Repeated bioactivity-directed chromatographic separation on silica gel columns resulted in the isolation of three compounds: 4-phenyl-2-butanone, 1,4-cineole, and nerolidol. 4-Phenyl-2-butanone shows pronounced contact toxicity against *S. zeamais* (LD_{50} = 6.98 mg/adult) and was more toxic than either 1,4-cineole or nerolidol (LD_{50} = 50.86 and 29.30 mg/adult, respectively) against the maize weevils, while the crude essential oil had an LD_{50} value of 11.67 mg/adult. 4-Phenyl-2-butanone and 1,4-cineole also possessed strong fumigant toxicity against the adults of *S. zeamais* (LC_{50} = 3.80 and 21.43 mg/L), while the crude essential oil had an LC_{50} value of 9.66 mg/L.

Liu et al. [44] found that *Artemisia capillaris* and *A. mongolica* possess insecticidal activity against the maize weevil, *S. zeamais*. The essential oils of aerial parts of the two plants were obtained by hydrodistillation and investigated by GC and GC-MS. The main components of *A. capillaris* essential oil were 1,8-cineole (13.75%), germacrene D (10.41%), and camphor (8.57%). The main constituents of *A. mongolica* essential oil were α-pinene (12.68%), germacrene D (8.36%), and γ-terpinene (8.17%). Essential oils of *A. capillaris* and *A. mongolica* possess fumigant toxicity against *S. zeamais* adults with LC_{50} values of 5.31 and 7.35 mg/L, respectively. The essential oils also show contact toxicity against *S. zeamais* adults, with LD_{50} values of 105.95 and 87.92 mg/adult, respectively.

The essential oil of *Atractylodes chinensis* (DC.) Koidz was found to possess strong insecticidal activity against the common vinegar fly, *Drosophila melanogaster* L. [45]. The essential oil was extracted via hydrodistillation, and its constituents were determined by GC-MS analysis.

The main components of *A. chinensis* essential oil were β-eudesmol (21.05%), β-selinene (11.75%), γ-elemene (7.16%), and isopetasam (5.36%). Bioactivity-directed chromatographic separation on repeated silica gel columns led to the isolation of five compounds: atractylon, α-elemol, β-eudesmol, hinesol, and β-selinene. β-Selinene, α-elemol, and hinesol showed pronounced contact toxicity against *D. melanogaster* adults, with LD_{50} values of 0.55, 0.65, and 0.71 μg/adult, respectively. Atractylon and β-eudesmol were also toxic to the fruit flies (LD_{50} = 1.63 and 2.65 μg/adult, respectively), while the crude oil had an LD_{50} value of 2.44 μg/adult.

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