Chapter

Citrus Essential Oils: A Suite of Insecticidal Compounds

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Abstract

Citrus essential oils (CEOs) and their constituent compounds are being reported to have multifarious activities. In this chapter an attempt is made to discuss the insecticidal activities, as well as CEO profile of different vegetative part of *Citrus* species and biocidal potentiality of their constituent compounds against diverse insect pests. It is observed that in most of the CEO constituent profile, limonene is the major constituent compound. Other important constituents present in different percentages in different CEOs are β-citronellal, linalool, pinene, β- caryophyllene, β-myrcene, terpinene, citral etc. These plant EO constituents are reported to have insecticidal effects against diverse insect species. Taking the four peel EOs of *Citrus limon, Citrus paradisi, Citrus medica, Citrus maxima* commonly grown in North Eastern part of India, study on their insecticidal effects against *Dolichoderus affinis* (Hymenoptera: Formicidae) was made and result is presented showing higher fumigant toxicity of *C. medica* and *C. limon* oil against the ant sp. With the increasing awareness for using safe insecticidal products among consumers, the citrus EOs with their attracting terpene compounds having good insecticidal potency bear all attributes to be used as commercial green pesticides in coming days both in indoor and outdoor management of insect pests.

Keywords: essential oils, limonene, *Dolichoderus*, *Citrus medica*

1. Introduction

The genus Citrus has tremendous industrial value all over the globe not only for its nutritive juicy high valued fruits but also for the essential oils present in its different vegetative parts. Thus, both the Citrus fruits and citrus essential oils bear potential to generate livelihood & to boost the country's economy. Citrus essential oils (CEOs) with diverse biologically active compounds of terpene groups with pleasant aroma have already achieved significant positions in flavor, food, cosmetic industries. At the same time, because of their antimicrobial activities as well as anticancer, antioxidant, anti-inflammatory, metabolic disorder alleviating activities etc. these oils and their compounds have been getting importance in pharmaceutical and medical sectors for the last few decades [1]. A good number of studies also reported insecticidal potential of citrus EOs extracted from different citrus sp. and their constituents at different times, a few of which are commercialized to be used by the consumers against insect pests. There are 33 recorded species of citrus worldwide (ThePlantList.org) with many recorded and unexplored varieties present in different parts of the world. The essential oil profile of different citrus species varies although some of the constituent compounds are common but present in different amounts in the total bulk oil. Even the oil profile of different vegetative parts of a single citrus species are not identical. Understanding of essential oil profile of diverse citrus species grown in wild, semi wild and cultivated state across the globe at different seasons is the much-needed task as the quality of the oil, oil yield percentage, consistency of the constituents even varies with seasonal changes, geographical location, harvesting time of the plant parts, soil type etc. however from the existing GC–MS profile of different *Citrus* sp. reported at different times and from different places, it is apparent that two -three dominant compounds are mostly present in most of the Citrus species. The literature revealed that the Citrus EO comprises more than 200 compounds of which 85–99% are volatile and 1–15% nonvolatile compounds. The volatile compounds comprise mostly monoterpenes (predominant limonene), some sesquiterpenes and their oxygenated derivatives [2].

Pest control sector is dominated by synthetic pesticidal products for many decades. At recent times with increasing concern to ecofriendly product, plant essential oils are getting renewed interest as they are not only effective but also comparatively safe and environment friendly in comparison to synthetic counterparts. Essential oils are part of natural plant defense system and many of them are proved effective and some are exploited for integrated management practices of pest and pathogens. As some citrus species are naturally resistant to certain group of pests and or pathogens, it is assumed that certain bioactive compounds may present in the essential oil part of those citrus species. It is already established that citrus essential oils of different citrus species are effective against wide range of pest and pathogens. It is also important to have an insight about the interaction of citrus constituents against its own insect pest and pathogen complex to be used as insecticidal, repellent and bactericidal etc. A few papers highlighted beneficial effects of using citrus essential oil against its own pest and pathogen complex. The added advantage of considering CEO as insecticidal and insect repellent is that the plant is edible therefore safe for residual contamination or toxicity to consumer. At the same time the pleasant aroma offers consumer acceptance.

2. Citrus EO against insect sp

CEO and extracts have been tried against a wide range of insect pests for assessing their insecticidal as well as repellent properties. In some parts of the world citrus plants have been traditionally used to ward off a insect pests. Some recent reports especially of the last two decades of the insecticidal and repellent effects of different citrus sp. are presented below. Most of the works were carried out on dipteran, lepidopteran, hemipteran and coleopteran insect pests.

Topical toxicity of the essential oil of *Citrus hystrix* with LD50 of 26.748 μL/g and antifeedant properties leading to severe growth inhibition has been reported against tobacco armyworm *Spodoptera litura* [3]. The fumigant toxicity and repellent effect of the n-hexane extract of the plant leaf was documented against stored grain pest *Lasioderma serricorne* [4]. Fumigant toxicity of peel oils of lime, orange, mandarin, tangerine, grapefruit and lemon were reported against three store grain pest species *Callosobruchus maculatus, Sitophilus zeamais and Dermestes maculatus* [5].

The peel essential oil of the plant is reported to possess repellent effect against *Callosobruchus maculatus* [6], *Aedes aegypti* and *Anopheles minimus* [7]. Similarly, the insecticidal and repellent activity of *Citrus reticulata*, *Citrus limon* and *Citrus aurantium* peel oils was demonstrated against *Callosobruchus maculatus* [8]. Insecticidal activity of *Citrus limon* and *Citrus sinensis* against vine mealybug, *Planococcus ficus* [9]. The larvicidal and adulticidal effects of *Citrus limon* and

Citrus sinensis are mentioned against *Attagenus fasciatus* and *Lasioderma serricorne* [10]. The seed and peel extracts of *Citrus limon* L. was reported to have the highest larvicidal toxicity (LC50 values of 395.59 ppm for seed; 468.69 ppm for peel) after 24 hours over EOs of *Citrus grandis, Citrus sinensis, Citrus paradise, Citrus reticulate* [11]. Essential oil of *Citrus reticulata* and *Citrus sinensis* was reported effective against the fourth instar larvae and adults of *Tribolium castaneum* with higher potency of *Citrus reticulata* [12].

The seed EOs of *Citrus reticulate* var. kinnow, *Citrus reticulate* var. freutrall, *Citrus sinensis* and *Citrus jambhiri* was tested against *Tribolium castaneum* with promising efficacy in terms of LC50 for *Citrus jambhiri* followed by Citrus reticulate and *Citrus sinensis* [13]. Similarly Oboh et al. [14] recorded insecticidal efficacy of *C. sinensis* peel essential oil against Callosobruchus mamulatus, *Tribolium confusum*, *Sitophilus oryzae* with LC50 value of 21.8, 38.9, 60 μl/l.

Comparative evaluation of toxicity of EOs of *C. limon, C. aurantifolia, C.sinensis* in filter paper impregnation method showed highest toxicity of *C. limon* (95% mortality) followed by *C. aurantifolia* (92.5%) and *C. sinensis* (82.5%) against carpenter ant *Camponotus nearcticus* [15]. But, Guerra et al. [16] comparatively lower topical toxicity (15% mortality) of *C. limon* EO against *Camponotus pennsylvanicus* among the eleven different EOs tested. Essential oils and or extracts of *C. maxima* or *C.grandis* have been reported effective against different mosquito species. In our earlier studies we recorded differential biocidal activities of essential oil extracted from peel and leaf part of *Citrus grandis* grown in Assam against different developmental stages of *Aedes aegypti* and *Culex quinquefasciatus* [17, 18]. EO extracted from leaves was found more effective against egg stage while oil from peel was recorded more effective against larval and adult stages of *A. aegypti* [18]. The leaf and peel oil of the plant was recorded highly effective against egg and larval stage with LC50 value of below 50 ppm but did not found much effective against adult stage of *Culex quinquefasciatus* although having repellent properties with good protection time [19]. In a recent study we observed synergistic larvicidal response of *Citrus grandis* leaf oil with *Allium sativum* bulb oil against *C. quinquefasciatus* [19]. Manorenjitha et al. [20] tested hexane, ethyl acetate, methanol, water and essential oil extract of *C. grandis* peel extract for evaluating oviposition deterrent and repellent properties on *Aedes aegypti*. They observed promising oviposition deterrent activity of ethyl acetate fraction (10 ppm concentration) in breeding plates kept within mosquito cage and effective repellency (94.7%) of 20% essential oil fraction of the peel while offering animal bait in modified tunnel test. A study for toxicity assessment on worker termites *Odontotermes feae*, essential oils of *Citrus grandis* with LC50 value of 273.36 ppm was found to show maximum toxicity out of *Citrus paradisi, Cassia fistula*, *Citrus grandis* EOs [21].

The peel essential oils of *Citrus aurantifolia* has been reported as insecticidal, repellent, and larvicidal against *Aedes aegypti* [22]. In our previous study, we observed the ovicidal, larvicidal and adulticidal effects of leaf and peel essential oil of *Citrus aurantifolia* against *Aedes aegypti* [23].

Promising fumigant toxicity of the peel EO of *Citrus aurantium* and *Citrus sinesis* from the north east Brazil with LC50 value of 5.80 μL/L of air and 3.80 μL/L of air respectively and oviposition deterrent activity at 3.5 and 7.0 μL/L of air against the whitefly *Bemisia tabaci* [24]. The same oil was reported effective against the larval and adult stages of tomato leafminer *Tuta asoluta* (Lepidoptera: Gelechiidae) [25]. The insecticidal activity of *Citrus aurantium* EO against adult housefly *Musca domestica* was reported next to the activity of EO of *Citrus sinensis* [26]. *Citrus aurantium* leaf EO was found as effective fumigant against sawtoothed grain beetle *Oryzaephilus surinamensis*, cigarette beetle *Lasioderma serricorne* and rice weevil *Sitophilus oryzae* with LC50 value of 64.94, 202.49 and 364.25 μL/L of air respectively [27]. Similarly, Bnina et al. [28] noted fumigant toxicity of peel, leaves and flowers essential oil of *Citrus aurantium* against four stored grain pests namely *Tribolium castaneum, Liposcelis bostrychophila, Sitophilus granarium, Cryptolestes ferrugineus* with LC50 value of 64.78%, 23.11%, 101.50% and 20.62% respectively. They also noted repellent property of the oil against these pests. Yazdgerdian et al. [29] tested contact and residual toxicity of eleven essential oils including *Citrus aurantium*, *Citrus sinensis*, *Citrus limon* against wooly beech aphid, *Phyllaphis fagi* (Hemiptera: Aphididae) and rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) and recorded highest residual toxicity (40%) of *C. aurantium* among the citrus sp. tested against the targeted species. Similarly, without affecting the seed viability of stored cowpea and consumer acceptability, peel EOs of *Citrus nobilis* and *Citrus medica* was reported to show significant reduction of egg laying, egg hatching, and adult emergence percentage of pulse beetle *Callosobruchus maculatus*. Both the oils showed dose dependent repellency with higher effect for EO of *C. nobilis* [30]. Like that of EOs, the nonpolar petroleum ether extract of ripe fresh fruit of *C. aurantium* was reported effective against adults of olive fruit fly *Bactrocera oleae* (Diptera:Tephritidae) in petri dish residual exposure test [31]. The same solvent extract also reported to have good toxicity against medfly *Ceratitis capitata* adults (LC50 value of 70.6 and 147.1 lg/cm^2 for male and female respectively at 96 h in Petri dish residual bioassay) [32].

Moravvej et al. [33] tested fumigant toxicity of EOs from four citrus species namely *C. paradisi, C. limonium, C. sinensis* and *C. aurantium* among which *C. paradisi* was the most effective with LC50 value of 125 μl/L and *C.sinensis* is the least effective with LC50 value of 269 μl/L against *Callosobruchus maculatus.* However, fumigant toxicity of *C.sinensis* was reported against *Solenopsis invicta* (Hymenoptera: Formicidae) with 100% mortality at 3 mg/tube after 24 hrs [34]. Ethanolic extract of the same plant was found effective against *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* with larval and adulticidal LC50 value of below 500 ppm along with more than 50% repellency at 150 ppm concentration till 180 min [35].

Ezeonu et al. [36] reported the insecticidal properties of the volatile peel extracts of *Citrus sinensis* and *Citrus aurantifolia* against mosquito, cockroach and housefly and recorded higher insecticidal potency of the peel extract of *Citrus sinensis* with maximum fumigant effect (85% at 60 min) against cockroach.

Zewde and Jembere [37] evaluated the solvent extract and essential oil of *Citrus sinensis* against *Zabrotes subfasciatus* (Coleoptera: Bruchidae) for their repellent, fumigant and protectant properties and recorded no progeny emergence on application of oil at low dose (30 mg of EO), prominent percent mortality at high dose (750 mg of essential oil killed 67% of Z. subfaciatus after 96 hours). Fumigant toxicity of *C. sinensis* EO was also reported effective against second instar larvae of *Musca domestica* (Diptera: Muscidae) with LC50 values of 71.2 μl/L as well as percent inhibition of pupae with 46.4% at 40 μl/L exposure concentration [38].

Orange oil extract was also recorded effective against the subterranean termite *Coptotermes formosanus* (Isoptera: Rhinotermitidae). Application of the oil extract at 5 ppm concentration resulted in 96% and 68% mortality respectively in closed container. At the same time termites did not show tunneling behavior on the 0.2 & 0.4% oil treated soil [39].

Majeed et al. [40] reported the insecticidal activity of the acetone, ethanol and aqueous extracts of seeds, leaves and fruit peels and leaves of *Citrus aurantium* and *Citrus sinensis* and two other plants against mealy bug *Drosicha mangiferae* (Hemiptera; Pseudococcidae) of which ethanol extracts of *C. sinensis* seeds and *C. aurantium* leaves were suggested as considerably toxic against the said insect.

C. medica peel essential oil was reported as more effective in filter paper fumigation method against stored grain pest *Tribolium castaneum* (Coleoptera) with LC50 value of 29.5 mg/L air than the leaf EO [41].

Abdel-Kawy et al. [42] showed *Citrus trifoliata* essential oil loaded nano-cubosome significantly enhanced insecticidal property of the essential oil against the second instar larvae of *Spodoptera littoralis*.

While working as biocidal and repellents, plant products including EOs and constituent terpene compounds are reported to act on cholinergic system [43], voltage – gated sodium channel of the nerve membrane, glutamate-gated chloride channel [44], GABA-system [45], Octopaminergic system [46], mitochondrial system [47], endocrine system disrupting the endocrinological balance and respiratory system of insect body. However, not much studies yet conducted on detailed study on the mode of action of EOs and their constituent compounds.

3. GC-MS profile of citrus EO

With the development of GC–MS technique, profiling of essential oil became easier. The composition of different citrus species from different parts of the world have been reported utilizing this technique. Most of the profiling results although detected average 20–50 numbers of compounds, a few compounds mostly occupy the major share of the bulk oil. The dominating compounds in most citrus species is limonene. In some species like *C. hystrix* the major compound is β-citronellal. The other common constituent compounds observe to be present in many GC–MS results of citrus species are linalool, pinene, β- caryophyllene, β-myrcene, terpinene, citral etc. Some of the reported constituent profiles of citrus species are mentioned below.

3.1 *Citrus hystrix*

From the leaf essential oil of *C. hystrix* in Malaysia, 29 compounds were reported, out of which beta-citronellal was the major compound (66.85%) [3]. The other compounds present in more than 1% total compositions were β-citronellol (6.59%), linalool (3.90%), 5,9-dimethyl-1-decanol (4.96%), methyl citronellate (1.90%), geranyl acetate (1.80%), citronellol (1.76%), 3-undecanol (1.04%). The same compound (beta-citronellal) with 86.43% amount was reported in another study from Indonesia along with 11.48% citronellol and 1.65% β-linalool [4].

3.2 *Citrus maxima* (synonym *Citrus grandis*)

In the leaf EO of *C. maxima*, 42 constituent compounds were reported to be present with citronellol as the major compound (28.26%) of the essential oil. The compounds comprised more than 1% of the oil compositions were β- caryophyllene (16.89%), –spathulenol (9.32%), α-caryophyllene (2.48%), γ-cardinol (3.16%), α-cardinol (2.51%), 2n-hexylcyclopentanone (2.22%), caryophyllene oxide (1.03%). Again, 34 compounds were identified from the peel essential oil of the same plant with limonene comprising bulk of the oil constituents (89.04%) [48]. The major compounds identified in more than 1% of the total composition were β-pinene (2.25%), β-myrcene (2.06%), β- copaene (1.76%). Compounds comprising more than 0.3% amount but below 1% were linalool, β-phellandrene, α-pinene, terpinene-4-ol. Earlier 35 compounds with limonene as the major constituent compound (93.2%) was reported from the fruit peel oil [49]. Myrcene comprised 2.9%

and other six compounds namely α-pinene, octyl acetate, germacrene -D, linalool, decanal, geranial comprised above 0.2% but below 1% of the total composition.

3.3 *Citrus aurantium*

Phytochemical profiling of essential oil of *C. aurantium* showed the presence of 25 compounds with limonene occupying 87.52% followed by linalool (3.365%) and β-myrcene (1.628%) as dominant compounds [25]. From Tunisian *Citrus aurantium*, limonene percentage in leaves, flowers and peel EOs were 6.52, 5.03, 73.6% respectively, linalool occupied 37.24, 41.82, 4.8% respectively, linaly acetate occupied 7.87, 13.75, 1.6% respectively and neral share was almost of similar (3.40, 4.80, 3.26% respectively) percentage. β-pinene composition for leaves and flowers were 9.68 and 9.21% respectively and α-thujene composition were 10.65 and 6.15% respectively but both β-pinene and α-thujene present below 0.5% in peel EO of the plant [28].

3.4 *Citrus aurantifolia*

In our recent studies 31 compounds from the leaf oil and 26 compounds from the peel essential oil was recorded from GC–MS analysis of EO of *Citrus aurantifolia* in India. Citral and limonene were noted as the major constituent compound of leaf oil and limonene and palatinol-1C as the major constituent of peel essential oil of the plant [19, 23]. The EO of the plant from Italy reported to comprise limonene (53.8%), γ-terpinene (16.5%), β-pinene (12.6%), β-Bisabolene (1.33%), Geranyl acetate (1.06%), Neryl acetate (1.12%), Geranial (1.84%), sabinene (1.74%), and α-Pinene (1.97%) [50]. Phytochemical analysis of leaf and peel EOs of the plant from Brazil [51] showed limonene as the dominant compound in both leaf (32.7%) and peel (77.5%) part. Other prominent compounds in the leaf EO were linalool (20.1%), citronellal (14.5%), citronellol (14.2%), trans-β-Ocimene (2.7%), geranial (2.6%), neral (2.1%), trans-β-Caryophyllene (2%), myrcene (1.4%) etc. The other major compounds of leaf EO were myrcene (4.4%), linalool (3.5%), citronellal (3.2%), citronellol (2%), β-Bisabolene (1.5%) etc.

3.5 *Citrus sinensis*

Phytochemical analysis of peel essential oil from three varieties of *C. sinensis* from Kenya showed presence of 56 components in Salaustiana variety, 73 in Valencia and 72 in Wshington varirty. Limonene occupied more than 90% in all the essential oil (Salustiana 94.6%, Valencia 92.5% and Washington 90.5%); alpha terpinene occupied 1.5% in Valencia and Washington and 1.7% in Salustiana [52]. Limonene with 90% share and β-myrcene, γ-terpine, linalool with 1.88%, 1.21% and 0.88% share out of 32 identified compounds in peel EO of *C. sinensis* has been reported from China [53]. GC–MS analysis of the plant EO from Argentina showed limonene (92.47%), linalool (1.43%), and β-myrcene (0.88%) as the major constituent compounds along with terpineol (0.28%) in lesser amount [26]. Almost similar constituents were identified from EO of *C. sinensis* with D-limonene (65.28–80.18%), Linalool (0.32–2.20%) and β- pinene (1.71 5.58%) as major part in another study [34].

3.6 *Citrus nobilis*

Phytochemical study on constituents from the peel of *C. nobilis* from China showed D- limonene (12,601 μg/g) as the major constituent compound followed by β-myrcene (1600 μg/g), β-pinene (82.64 μg/g), p-mentha-1,8-dien-3-one (41.33 μg/g), α-pinene (25.41 μg/g), geranial (21.32 μg/g), sabinene (21.18 μg/g), E-β-ocimene (14.97 μg/g), linalool (10.38 μg/g), α-terpineol (6.36 μg/g). Other compounds are present in lower amounts (below 5 μg/g) [54]. In another study from Sri Lanka, 37 compounds were reported from peel part of which D-limonene (45%) was the major one followed by cyclopentane-2-methyl-1-methylene-3-(1-methylethenyl) (3.94%), p-mentha-4,8-diene (3.73%), α-terpinolene (3.03%), methyl-2-(methylamino) benzoate (2.4%), α-fernesene (1.1%). Other compounds were present in below 1% [30].

3.7 *Citrus limon*

Phytochemical analysis of citrus leaf EO from Iran showed presence of 27 compounds of which the major compound was linalool (30.62%). The other compounds present in significant amount were geraniol (15.91%), α-terpineol (14.52%), linalyl acetate (13.76%), geranyl acetate (6.75%), B-pinene (4.51%), neryl acetate (4.24%), p-Cymene (1.86%), and limonene (1.13%) [55]**.** Chemical composition of EO of *C. limon* grown in Iraq [56] showed presence of 24 compounds with limonene as principal compound with 29.52% share. Other major compounds were β-Pinene (23.89%), α-Pinene (2.25%), Myrcene (1.31%), (Z)-β-Ocimene (2.09%), Linalool (1.41%), (R)-Citronellal (15.10%), α-Citronellal (3.57%), (+)-α-Terpineol (1.57%), Neral (Z-Citral) (1.19%), Geranial (E-Citral) (1.73%), Thymol (9.79%), Citronellyl acetate (1.87%), Caryophyllene (1.36%), Phytol (1.36%). The analysis of the essential oil of *Citrus limon* from North-East India reported presence of 43 constituent compounds of which limonene (55.40%), neral (10.39%) trans-verbenol (6.43%) and decanal (3.25%) were the major constituent compounds [57].

3.8 Citrus paradisi

Phytochemical analysis result of *C. paradisi* peel EO from Turkey demonstrated presence of 25 constituent compounds of which limonene occupied the highest percentage (88.6%) of the oil. The other major compounds were α-terpinene (1%), and β-pinene (1.2%) [58].

From Nigeria, fifteen phytochemical constituents of the plant oil were reported. Among the compound limonene (94.2%) occupied the major share [59].

3.9 *Citrus medica*

A total of 19 constituent compounds were identified from leaf essential oil of *Citrus medica* from Bangladesh of which erucylamide (28.43%), limonene (18.36%), citral (12.95%), Mehp (8.96%), 2,6-octadien-1ol,3,7-dimethyl-acetate, (Z) (5.23%) were the major compounds. From peel essential oil 43 compounds were reported out of which isolimonene (39.37%), citral (23.12%), limonene (21.78%) were the major constituents. Three other compounds namely β-myrcene, neryl acetate and neryl alcohol were reported to present at around 2% each in total composition and remaining compounds were present in traces amount [60]. In a study carried out by Li et al. [61], all total 28 compounds were reported to present in the fruit essential oil of *Citrus medica* of which limonene (45.36%), γ-terpinene (21.23%), dodecanoic acid (7.52%) were documented as major constituent compounds. Compounds like β-bisabolene, tetradecanoic acid, α-terpineol, terpinene-4-ol, hexadecenoic acid, α-bergamotene, α-pinene, β-pinene comprised between 5–1% range in total composition. In another study fruit peel EO of the plant was reported to comprise limonene (38.7%), γ- terpinene (28%) and o-cymene (15.2%) as major compounds [41].

4. Citrus EO compounds against insect sp

Essential oil composition of different citrus sp. across the globe although may vary but some of the compounds are observed as common in most of the oil profile. The most dominating and commonly present compound is limonene. Other common compounds are citronellal, citronellol, linalool, pinene, myrcene, ocimene, terpinene, caryophyllene etc. The bioactivity of EOs is often related to the activity of major compounds present in the crude oil and some of the studies have already established this fact. Individual assessment for insecticidal property of these common constituent compounds have been performed by different researchers and some of them were found active against insect pest. Limonene and other *Citrus limon*oids are reported as insect repellents, feeding deterrents, growth disruptors, and reproduction inhibitors against a wide range of pest complexes. Insecticidal activity of limonene was reported effective against *Tuta asoluta* (Lepidoptera: Gelechiidae) [25]. Yoon et al. [62] revealed repellent property of different citrus oil and its major compound limonene against different species of cockroaches like *Blatella germanica, Periplaneta americana* and *Periplaneta fuliginosa.* However, Karr and Coats [63] did not get significant insecticidal activity of d-limonene against *Blattella germanica, Musca domestica, Sitophilus oryzae* and *Diabrotica virgifera virgifera.* In contrast they reported enhanced growth of nymph of *Blatella germanica* after oral administration of d-limonene.

In another study against cat flea species *Ctenocphalides felis* (Siphonaptera: Pulicidae), d-limonene (LD 50 against larvae, adults 226, 160 $\mu g/cm^2$ respectively) and d-limonene with piperonyl butoxide (PB) (LD 50 against larvae, adults 157, 49 $\mu g/cm^2$ respectively) were reported effective against all the life stages except the pupal stage of the flea species [64]. Fumigant toxicity of d-limonene, α- terpineol etc. also reported against honey bee *Apis mellifera* and tracheal mite parasite species *Acarapis woodi* [65].

Fouad and da Camara [66] extracted the essential oil from *Citrus aurantiifolia* and *Citrus reticulate* and analyzed the phytochemical constituents using GC–MS and found limonene as the major constituent compound, 38.9% of the *C. aurantiifolia* oil and 80.2% of the *C. reticulata* oil. They analyzed the enantiomers of limonene against the said insect. They found that *Citrus reticulate* was more toxic than *Citrus aurantifolia* towards the said insects. (R)-limonene was shown to have greater toxicity against *S. zeamais* than the (S)-limonene as found in the ingestion bio assay. Repellent bioassay showed (S)-limonene more susceptible to *S. zeamais* than (R)-limonene.

After identifying limonene as major compound in the EOs of *Citrus aurantiifolia* (38.9%) and *Citrus reticulate* (80.2%) Fouad and da Camara [66] tested enantiomers of limonene against *Sitophilus zeamais* and recorded greater toxicity of (R)-limonene than the (S)-limonene in the ingestion bio assay. But in the repellency test they found more susceptibility of *S. zeamais* towards (S)-limonene than (R)-limonene.

In a recent study, Sowler et al. [67] comparatively evaluated the effect of laboratory grade limonene and a commercial limonene-based insecticide against *Haematobia irritans irritans* in terms of deterrence, mortality, and reproduction. They showed that the egg viability was decreased in both the treatment, however, commercial limonene that caused loss of viability at 5.8% concentration was ovicidal in case of laboratory grade limonene. However, in terms of knockdown effect commercial limonene was better. Interestingly, at a concentration of less than 0.1%, both the commercial and laboratory grade limonene were acted as attractant.

Giatropoulos et al. [68] tested essential oil of *Citrus sinensis, Citrus limon,* and *Citrus paradise* and their constituents and recorded γ- terpinene as the most toxic compound against *Aedes albopictus* larvae. They also reported that the constituent compound tested for repellency were better mosquito repellent than the parent essential oil. Similarly, Luo et al. [41] analyzed composition of leaf and peel essential oil of *C. medica* and tested both crude oil and major compounds viz. limonene, terpinene, o-cymene, β-caryophyllene against *Tribolium castaneum* and recorded γ-terpinene as the most effective insecticidal compound having LC50 value of 4.1 mg/l air and β- caryophyllene as the effective repellent compound. Limonene was reported to have almost similar fumigant toxicity like that of crude EO of *C. aurantium* against *Tribolium castaneum, Sitophilus granarium* and *Cryptolestes ferrugineus* [28]. In our earlier studies we found higher toxicity of citral, that is the major compound of the essential oil of *Citrus aurantifolia* than the crude oil as mosquito larvicidal, ovicidal and adulticidal against *Aedes aegypti* [23]. Plata-Reuda et al. [69] reported the insecticidal activity of citral and geranyl acetate against peanut beetle *Ulomoides dermestoides*. These compounds affected the survivorship, locomotor activity and reduced the respiration rate of the said species.

Nootketone and carvacrol, a phytochemical constituent present in essential oil of Citrus [70] acts as insecticidal compound against *Aedes aegypti* [71]. Pajaro-Castro et al. [72] recorded the neurotoxic effects of linalool and β-pinene on *Tribolium castaneum*. They observed that at low concentration both the compounds were attractant towards the insect and at higher concentration the compounds were repellent. Individual treatment of limonene and linalool was found to have fumigant toxicity against two ant species namely *Acromyrmex balzani* and *Atta sexdens* with LC50 values of 5.72 μl/L, 5.38 μl/L and 2.40 μl/L, 5.34 μl/L respectively [73]. Similarly individual treatment of β-caryophyllene is reported to have good contact toxicity against these two ant sp. but with low fumigant toxicity [74]. Fumigant toxicity of limonene, linalool and β- pinene were also reported effective against fire ant *Solenopsis invicta* (Hymenoptera: Formicidae) [34].

Linalool, α-terpinene was reported to show 100% fumigant toxicity against adult rice weevil *S. oryzae* at 3.9 mg/L [75].

Muller et al. [76] recorded 85.4%, 71.1%, and 29% repellency of the candles prepared with 5% geraniol, 5% linalool and 5% citronella against mosquitoes on human landing bioassay. They observed similar repellency against sand flies too. 78% repellency of *Culex pipiens pallens* was reported after using 30% citronellal [77]. Progeny deterrent, antifeedant, egg hatching inhibition activity was documented after application of 5–10 μl citronellol against *Callosobruchus analis* [78]. Compounds like limonoids act as synergists to enhance the activities of other biological and or synthetic insecticides [79]. We recorded medium larvicidal and adulticidal potential of limonene against *Aedes aegypti*, but found higher toxicity when combined with diallydisulphide and carvone respectively [80]. So, it is not always the individual compound that act as the most active but appropriate combinations of compounds having synergistic effects would be more fruitful as insecticidal against insect pests.

5. Citrus of North East India

North East India is enriched with Citrus species having documented 23 species and 68 varieties out of the 27 species of Citrus found in India [81, 82]. It is established that some of the citrus species are endemic and some are in endangered status [83]. According to Hore and Barua [84], there are eight citrus species indigenous to this region scattered in the form of semi-wild, wild state and some in cultivated state. Some of the species are naturally tolerant to viral and bacterial diseases and also for drought, cold and rainfall. For instance, *Citrus limon* is reported to resist scab, canker and gummosis, *C. indica* resistant to greening disease [84]. Due to diverse ecogeographical conditions the Citrus species and varieties of this region may bear specific traits including its aroma and essential oil constituents which needs to be investigated. However citrus crop and citrus essential oil-based industry is not yet flourishing in this part of the country. The information regarding Citrus essential oils extracted from the Citrus species grown in this particular area is relatively scanty. As the Citrus plants are rich in secondary metabolites to naturally defend an array of pathogens and pest complexes, it is expected that some of the key compounds for controlling insect pest may lie within the secondary metabolite compounds especially in the diverse aromatic essential oil part of the plants at least in the resistant citrus species.

Here we have attempted to evaluate insecticidal properties of essential oil extracted from the fruit peel of four citrus species namely *Citrus limon, Citrus maxima*, *Citrus paradisi* and *Citrus medica* grown in North Eastern part of India against one of the household ants *Dolichoderus affinis* (Hymenoptera: Formicidae). Fruits of *Citrus limon*, *Citrus paradisi* and *Citrus medica* were collected from Udalguri district, Assam (26.7210° N, 91.9906° E) and *Citrus maxima* from a daily market at Guwahati, Assam (26.1445°N, 91.736°E), in September, 2020. Essential oils from fresh peels were extracted by hydro-distillation using Clevenger's apparatus. After 6 hours, essential oils were collected, anhydrous sodium sulfate was added to absorb traces of moisture and were stored at 4°C till its use. The worker ants of *Dolichoderus affinis* from naturally existed colony located in the wooden frame of house wall were considered for the assessment. Fumigant toxicity of these oils were assessed following the method described by Hu et al. [34]. Six different concentrations viz. 0.25 μL, 0.5 μL, 1 μL, 2.5 μL, 5 μL and 7.5 μL. of each EO was individually loaded in 1.5 ml centrifuged tubes, evaporation of EOs was allowed by making five small holes of 1–1.2 mm diameter and placed into 500 ml properly cleaned borosilicate conical flask. Twenty worker ants were taken per flask in a replication and the flask was covered by aluminum foil and bound tightly by rubber band to prevent the loss of volatile compounds. For each concentration three replications were made. Equal number of controls set without oil were placed against each treatment. The environmental temperature range was 21–30°C and relative humidity range 56–99 during the experimental period. The Percent mortality [percent mortality = (Total no. of dead ants/Total no. of treated ants) × 100] data was recorded after 12 h and 24 h of treatment. The ants were considered to be dead if touched with a needle but did not show any movement. Based on the results sublethal concentration was determined using probit analysis with the help of SPSS and Minitab software. As shown in the figure, after treatment with *C. limon*, maximum 71.66% mortality was recorded at 12 h and 91.66% was recorded after 24 h at 7.5 μL treatment. The calculated LC50 for the oil was 2.66 μl / 500 air volume. For the EO of *C.paradisi*, maximum 15% mortality was recorded at 12 h and 66.66% mortality was recorded after 24 h of treatment. LC50 for the oil at 24 h was 7.32 μl / 500 air volume. For the EO of *C. maxima*, not more than 10% mortality was recorded even after 24 h at the highest dose applied and LC50 could not be computed for the oil. While for the EO of *C. medica*, maximum 56.6% mortality was recorded at 5 μl concentration at 12 h and maximum 88.33% mortality was recorded after 24 h at 7.5 μl. LC50 for *C. medica* at 24 h was recorded as 2.09 μl/500 air volume (**Figure 1**, **Table 1**). Highest toxic effect was recorded for *C. medica* followed by *C. limon*. Earlier Adusei-Mensah et al. [15] evaluated insecticidal properties of three citrus species viz. *Citrus aurantifolia*, *Citrus sinensis* and *Citrus limon* against *Camponotus nearcticus* (Formicidae) and recorded highest performance from *C. limon* with 95% mortality.

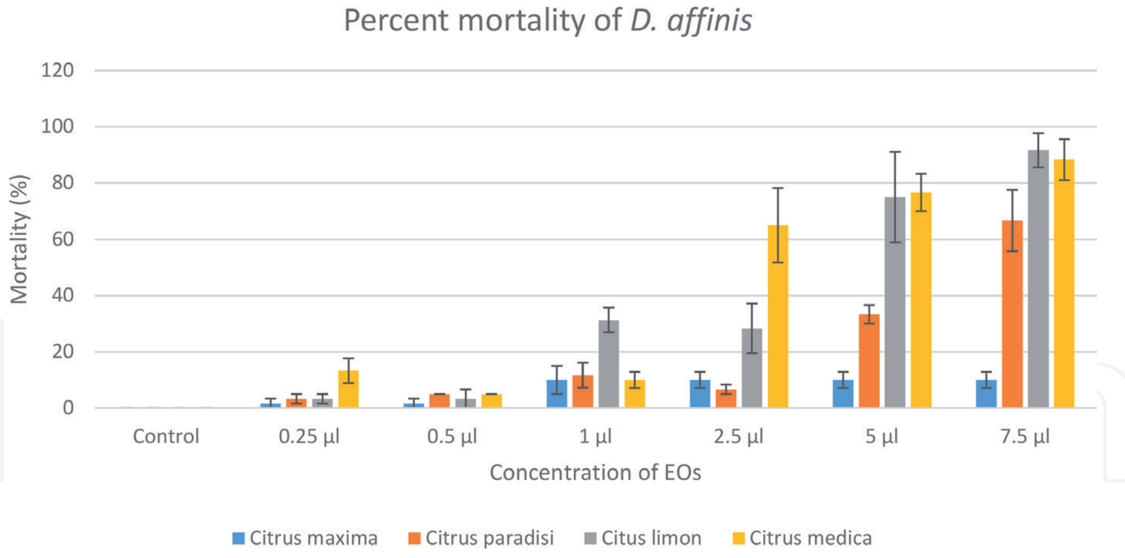

Figure 1.
Relation between concentration of EOs and respective percent mortality.

Essential oils	Time	LC50 value µl/500 ml air	95% confidence level		Regression equation	Chi-square value
			Lower limit	Upper limit		
Citrus paradisi	24 h	7.32	1.143	1.902	Y = 3.67118 + 1.53688X	31.750
Citrus limon	24 h	2.66	1.739	2.486	Y = 4.03677 + 2.26321X	49.452
Citrus medica	24 h	2.09	1.609	2.266	Y = 4.37759 + 1.94055X	52.747

Table 1.
LC50 values of the individual citrus oils against Dolichoderus affinis at 24 h.

But Guerra et al. [16] recorded only 15% mortality of *Camponotus pennsylvanicus* (Hymenoptera: Formicidae) on topical application of *C. limon*, the efficacy of which was comparatively lower than other eight EOs tested against the ant species. Not much studies on insecticidal activities of citrus EO against ants have been found to be reported. The findings showed the prospect of using *C. medica* and *C. limon* oil for controlling household ants.

6. Conclusion

With the increasing awareness of consumers for ecofriendly products and at the same time increasing resistance of insect pests against insecticides, the demand for novel, safe and effective products is increasing. As discussed above, the existing literature revealed presence of a good number of terpene compounds in different *Citrus* species which are present in different ratios although in most cases limonene is the predominant constituent. Both the crude oil as well as individual compounds possess good insecticidal and repellent properties against diverse insect pests, both indoor and outdoor. Our study also showed promising potential against *Dolichoderus affinis* while using four Citrus essential oils with higher efficacy of *Citrus medica* and *Citrus limon* essential oils. It is expected that in near future Citrus plant essential oils with their pleasant aroma and array of chemical compounds shall take leading space in development of insecticidal and repellent products to be used in both indoor and outdoor pest management practices against insect pests.

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