JURNAL ILMIAH AGRINECA
ISSN : 2721-074X (Online) - 2301-6698 (Print)
Homepage : http://ejournal.utp.ac.id/index.php/AFP/index

Review Article DOI : 10.36728/afp.v22i2.2008

Why *Eucalyptus Citriodora* Potential as Biopesticide ?

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ABSTRACT

Various efforts have been made to eliminate or at least reduce the negative impact of chemical pesticides that are currently widely used. One of these efforts is to use other effective control alternatives, such as bio-pesticides/botanical pesticides. *Eucalyptus citriodora* essential oil was previously better known in traditional medicine. However, many studies have shown that this essential oil has much potential to be used as a biopesticide. This article reviews the biopesticide potential of E. citriodora essential oil, including its herbicidal, bactericidal, fungicidal, nematicidal, insect-repellent, and insecticidal activities. E. citriodora oil contains citronellal, citronellol, 1,8-cineole, isopulegol, α-pinene, and citronellyl acetate. Citronellal and citronellol are one of the main toxins in E. citriodora essential oil. However, this review shows that the oil has a broad spectrum of biological activity, making it a simple and environmentally friendly pesticide.

KEYWORD
biopesticide, eucalyptus, insect, toxicity

INFORMATION
Received : 2 June 2022
Revised : 17 July 2022
Accepted :19 July 2022

Volume : 22
Number : 2
Year : 2022

1. INTRODUCTION

The use of chemical pesticides is currently being reduced or avoided. Although its use can supply food, prevent vector-borne diseases (like malaria), and give economic and labor benefits to the community, excessive use has driven users to explore alternatives. Uncontrolled and excessive use of chemical pesticides (currently used on vegetables and fruits) can contaminate water, soil, and other plants or living things, including beneficial insects, non-target plants, animals, and human health (Gyawali, 2018; Poudel *et al.*, 2020). These effects on humans can be rapid, such as poisoning that can result in death, or long-term, ranging from moderate consequences like mild skin irritation to severe ones like infertility, sexual function abnormalities, tumours, and even cancer (Aktar et al., 2009; Leoci and Ruberti, 2021).

Various efforts have been made to eliminate or at least reduce these negative impacts. These include using pesticides with the correct dose and concentration (according to regulations), using complete equipment when using pesticides (adopting the safety precautions) and starting to use other effective control alternatives such as the use of biopesticides/botanical pesticides.

Biopesticides have been used for many years because of their minimal risk to humans and the environment. Biopesticides are also known to have low levels of residue and persistence, a relatively high level of host specification, and a low potential to cause pest resistance. Several bioactive compounds play a role in each biopesticide or botanical pesticide (Figure 1) (Acheuk *et al*., 2022). Chemical insecticides are the most widely used pesticides in the world, as are botanical insecticides. These botanical insecticides affect insects in various ways depending on the active compounds contained in the botanical insecticides and the physiological characteristics of the insect. Some effects on these insects include repellent, feeding deterrents/antifeedants, toxicants, growth retardants, and attractants (Hikal *et al*., 2017). Some of these effects have been tested in several studies. One example is the repellent and toxicity effect of citronella grass (*Cymbopogon nardus*) essential oil on *Sitophilus oryzae* (Kardinan *et al*., 2021). Another example is an attractant effect of methyl eugenol produced from *Ocimum minimum* on fruit flies (*Bactrocera* spp.) (Kardinan and Maris, 2022).

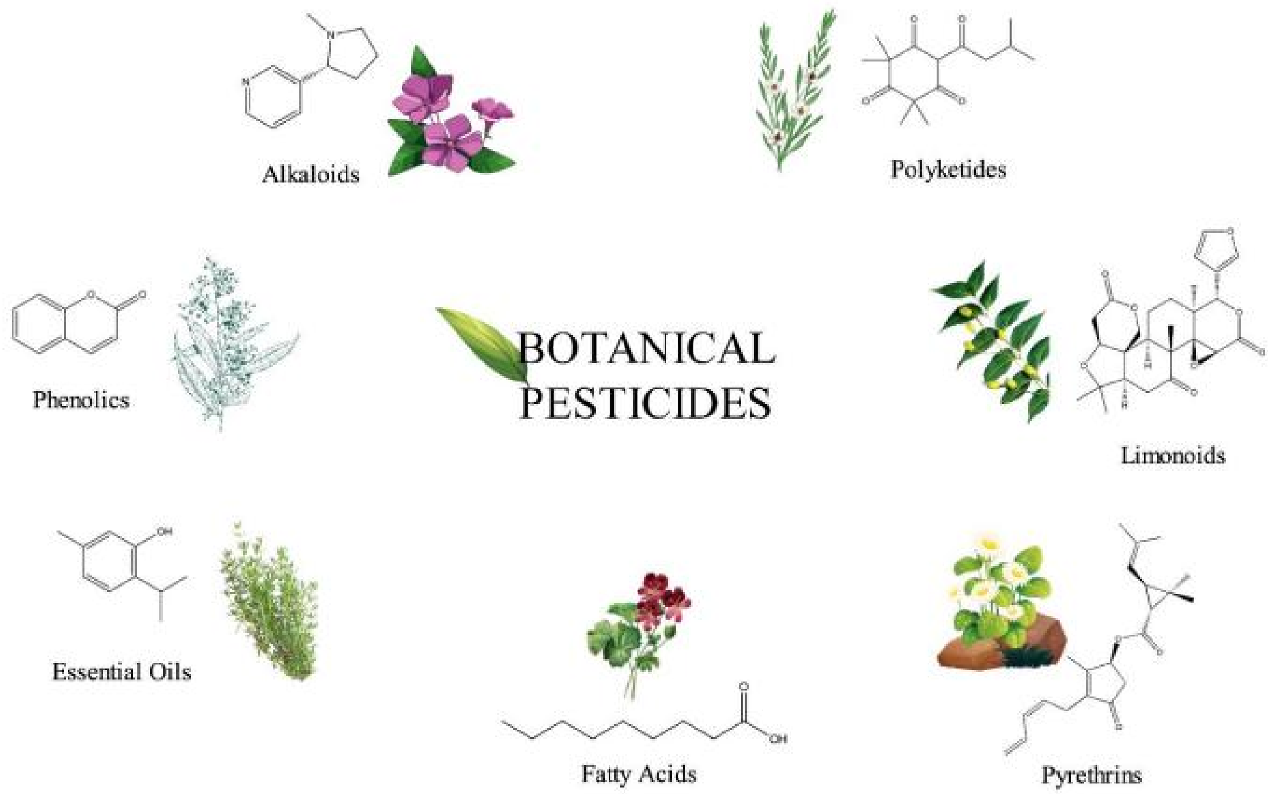

Figure 1. Bio active compounds in biopesticide (Acheuk et al., 2022)

Essential oils come from thousands of aromatic plants and can be obtained from various plant parts such as leaves, flowers, seeds, or roots, mainly through hydrodistillation or steam distillation (Fierascu et al., 2020; Acheuk et al., 2022). For example, 2.1% Eucalyptus citriodora essential oil was produced from 500 grams of fresh leaves, which were put into hydrodistillation for 2 hours using Clevenger-type equipment. The essential oil is then collected and dried over anhydrous sodium sulfate (Manh et al., 2020).

Essential oils are widely used as a mixture of cosmetics, food preservatives, and biomedicine. They are lipophilic, volatile, low-stable, water-insoluble, phytotoxic, and alter organoleptic characteristics. As an insecticide, essential oils can cause insect death in 2 ways, namely directly (neurotoxicity) and indirectly (dysfunction in biochemical and physiological effects) (Fierascu et al., 2020; Chang et al., 2022)

2. EUCALYPTUS PLANT

Several essential oils are often used as insecticides, including clove, rosemary, sage, and eucalyptus (Batish *et al.*, 2008; Chang *et al.*, 2022). *Eucalyptus* is a plant native to Australia and is considered a tall, green, majestic tree with about 700 species. It is also considered a popular cultivated plant in Brazil and Vietnam (Maciel *et al.*, 2010; Manh et al., 2020). These are some of the Eucalyptus species, namely *E. camaldulensis* (River red gum), *E. citriodora* (lemon-scented Eucalyptus), *E. globulus* (Tasmanian blue gum), *E. grandis*, *E. robusta*, *E. saligna*, *E. alba*, *E. tereticornis*, *E. cinerea*, *E. viminalis*, *E. polybractea* (blue mallee), and *E. urophylla,* where the essential oil from *E. citriodora* is considered one of the world's premier oils in terms of trade volume (Batish et al., 2008). Traditional African African societies have always used plants like eucalyptus to promote healing because traditional medicine is still dominant. The treatment method usually uses a decoction of eucalyptus leaves or directly. Eucalyptus leaves are usually used to treat asthma, bronchitis, tonsillitis, colds, and urinary and bleeding problems (Karemu et al., 2013).

Most essential oils from Eucalyptus species are produced and stored in secretory cells, so the leaves are odorous. Usually colorless or pale yellow, eucalyptus essential oil is rich in monoterpenes and sesquiterpenes. Some major components found in the leaves of Eucalyptus are citronellal, citronellol, 1,8-cineole, piperine, linalool, α-pinene, p-cymene, α-phellandrene, α-terpineol, limonene, and geranyl acetate (Barbosa et al., 2016). These various components of this eucalyptus essential oil usually have an effect synergistic to bring the whole pesticide activity (Batish *et al.*, 2008). This effect may be because when the detoxification system of the insect targets the significant component in the mixture, the minor component intoxicates the insect and is more toxic than when assayed alone (Manh et al., 2020).

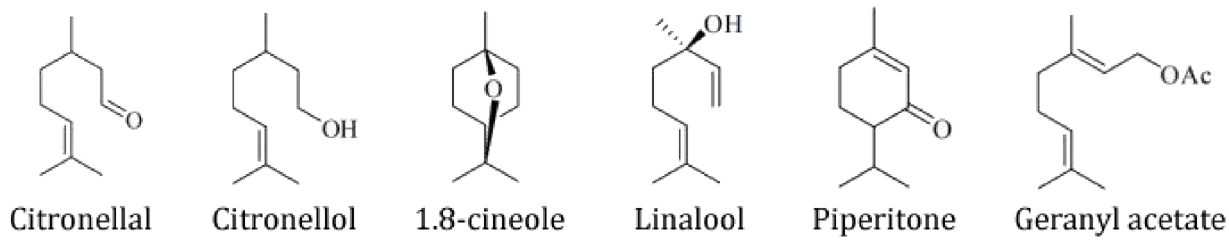

Figure 2. Some of the major constituents in eucalyptus essential oil (Barbosa et al., 2016)

Eucalyptus essential oil contains insecticidal, antifeedant, antifungal, antibacterial, herbicide, and nematicidal properties, like other botanical pesticides. Although the effectiveness of each of these functions also depends on the type and nature of the constituents. The effectiveness also depends on individual concentration (Batish *et al.*, 2008). The compounds in this essential oil can cause disturbances in biochemical and physiological functions in insects, which ultimately lead to insect mortality when ingested, inhaled, or absorbed by the insect's integuments. However, let us suppose the dose is not high enough to cause mortality. In that case, there is a possibility that the toxin can be neutralized by the insect's self-defense mechanism (through detoxification or insect metabolism) (Ataide *et al.*, 2022).

3. EUCALYPTUS CITRIODORA AS BIOPESTICIDES

E. citriodora is a large, fast-growing tree with smooth white bark and lemon-scented leaves. It is widely grown for extraction into an essential oil, usually rich in citronella, and is used in fragrances and as a flavoring agent (Salazar *et al.*, 2015). From the data in Table 1 below, it can be seen that the location of planting affects the types of main compounds and their percentages. However, other influencing factors include the environment, weather, climate, plant varieties, and the harvest stage (Benchaa *et al.*, 2018).

Table 1. The major compounds content of essential oils derived from the *E. citriodora* leaves

Origin	Major Compounds of *E. citriodora*
Argentina	citronellal (76.0%), iso-isopulegol (9.0%), citronellyl acetate (7.3%)
Australia	citronellal (68.9%), citronellol (7.6%), isopulegol (7.4%)
Benin	citronellal (52.8%), citronellol (20.0%), citronellyl acetate (9.0%)
Brazil	citronellal (94.9%), citronellyl acetate (2.6%), trans caryophyllene (2.5%)
Brazil	citronellal (89.6%), citronellyl acetate (3.3%), 1,8-cineole (2.9%)
Brazil	citronellal (82.3%), citronellyl acetate (7.8%), neothujan-3-ol (6.8%)
Brazil	citronellal (76.0%), neo-iso-3-thujanol (11.8%)
Brazil	β-citronellal (71.8%), (−)-isopulegol (7.3%), isopulegol (4.3%)
Brazil	citronellal (71.8%), isopulegol (4.3%)
Brazil	citronellal (71.1%), citronellol (8.8%)
Brazil	citronellal (67.5%), citronellol (6.9%), menthol (6.1%)
Brazil	citronellal (61.8%), isopulegol (15.5%), β-citronellol (7.9%)
Brazil	citronellal (64.9%), iso-isopulegol (10.2%), citronellol (8.3%)
China	citronellal (65.9%), citronellol (10.5%), 1,8-cineole (3.0%)
China	citronellal (55.3%), citronellol (8.3%)
Colombia	citronellal (49.3%), citronellol (13.0%), isopulegol (12.9%)
Colombia	citronellal (40.0%), isopulegol (14.6%), citronellol (13.0%)
Democratic Republic of the Congo	citronellal (72.7%), citronellol (6.3%), eugenol (3.5%)
India	citronellal (52.2%), citronellol (12.3%), isopulegol (11.9%)
India	citronellal (48.3%), citronellol (21.9%), iso-isopulegol (12.7%)
Indonesia	citronellal (90.1%), citronellol (4.3%)
Kenya	1,8-cineole (11.2%), β-pinene (3.2%), terpinen-4-ol (3.1%)
Pakistan	citronellal (22.3%), citronellol (20.0%)
South Korea	citronellal (73.0%), isopulegol (6.7%)
Taiwan	citronellal (49.5%), citronellol (11.9%), iso-isopulegol (10.4%)
Tunisia	1,8-cineole (54.1%), α-pinene (23.6%)

Source: Barbosa *et al.*, 2016

3.1. Herbicidal activity

When *E. citriodora* essential oil is applied in vapor form, it can significantly reduce the germination of *Parthenium hysterophorus* weed. This study shows that fumigation with eucalyptus oil can reduce water content, chlorophyll content, growth, and cellular respiration. The decrease in growth usually occurs more in broad-leaved weeds than grassy weeds. Weeds exposed to this fumigation can exhibit varying degrees of tissue damage, injury, chlorosis, necrosis, or withering. These treated plants usually show a higher rate of ion leakage, which indicates a loss of membrane permeability and ultimately causes severe crop damage (Batish *et al.*, 2008).

Research conducted by Benchaa *et al*. (2018) also showed a negative allelopathic effect of *E. citridora* essential oil on several types of weeds in Algeria, such as *Sinapis arvensis*, *Sonchus oleraceus*, *Xanthium strumarium* and *Avena fatua*. Seed germination and seedling growth were significantly reduced at lower concentrations (0.01%, 0.02%, and 0.03%). Meanwhile, at higher concentrations (3%), death (100% lethality percentage) of *S. arvensis*, *S. oleraceus* and *A. fatua,* and severe injuries on *X. strumarium* occurred. Symptoms of leaf chlorosis, necrotic spotting and drying of plants were seen on days 1–6 after treatment. The concentration of *E. citriodora* essential oil increased, and the symptoms became more significant and clearer. Six days after treatment at 1% of *E. citriodora* essential oil, the chlorophyll was reduced by 18.09 (*X. strumarium*), 38.74 (*S. arvensis*), 48 (*S. oleraceus*), and 70.39% (*A. fatua*). Electrolyte leakage per leaf disc increased with the concentration of essential oils. Significant reduction of membrane integrity also occurs due to an intense ion leakage by the concentrations 2 and 3%.

Bioassays in the two studies above showed that the oil revealed an herbicidal impact on seed germination, seedling growth, and sanitary plant state by reducing chlorophyll content and increasing membrane electrolyte leakage. *E. citriodora* essential oil has the potential to be used as a bioherbicide.

3.2. Bactericidal and fungicidal activity

Tests conducted by Salem *et al*. (2018) showed that the MIC (minimum inhibitory concentration) values of *E. citriodora* leave essential oil ranged from 0.06 mg/mL against *Escherichia coli* (Gram-negative bacteria) to 0.20 mg/mL against *Staphylococcus aureus* (Gram-positive bacteria). Those values were lower than the MIC values of streptomycin (negative control). It also showed a visible effect against the phytopathogenic bacteria such as *Dickeya solani*, *Pectobacterium carotovorum*, and P*ectobacterium atrosepticum*, which cause many diseases in potato production.

This study also showed that *E. citriodora* essential oil showed higher antifungal activity than the positive control. It even indicated that *E. citriodora* essential oil showed more potency than *C. macrocarpa* essential oil against several fungi tested, such as *Aspergillus flavus*, *Aspergillus ochraceus*, *Candida albicans*, *Fusarium oxysporum*, and *Penicillium funiculosum* (Salem *et al*., 2018). The above results show that *E. citriodora* essential oil has a bactericidal and fungicidal activity that can be used as a biopesticide to control plant pathogens.

3.3. Nematicidal activity

There was a test on *Haemonchus contortus*, gastrointestinal nematode parasitism that attacks sheep and goats. The *E. citridora* used in this study contained 63.9% citronella and was shown to affect different life stages (ovicidal and larvicidal effects) of *H. contortus.* This indicates that *E. citriodora* also has the potential to be an alternative anthelmintic (de Araújo-Filho *et al*., 2018).

3.4. Insect-repellent activity

Citronella oil is well known for its natural mosquito repellent properties (Manh *et al*., 2020). This certainly makes *E. citriodora* which has citronella as one of the main compounds, also suspected to have a repellent effect on insects. Several studies have proved this. One of them was a test conducted by Machingura (2019), which showed that *E. citriodora* leaf powder had a repellent effect against *Prostephanus truncatus* and *Sitophilus zeamais*. *E. citriodora* produces a very pungent odor even without being squeezed. This test showed that after 24 hours, it produced the highest repellency against *P. truncatus* and *S. zeamais*. The insects prefer to attack the untreated control rather than the treatment containing eucalyptus leaf powder. Even when a new *P. truncatus* is placed in the treatment area. It remains immobile for some time. This shows that when *P. truncatus* has a choice, they prefer to avoid the treated materials.

Tests on *S. zeamais* were also carried out by Karemu *et al.* (2013). The test showed that the essential oils of *E. citriodora* presented a repellent activity of 69.15%. In this study, the compounds that were successfully extracted from *E. citriodora* is 1,8-cineole (11.2%), spathulenol (6.9%), a-terpineol (3.4%), b-pinene (3.2%), 4-terpineol (3.1%) and a-pinene (2.2%). In these assays, 1,8-cineole has the strongest repellent activity, thus making a significant contribution to the repellent activity of *E. citriodora*.

Tests on cockroaches also proved that *E. citriodora* had a repellency effect, although not as high as *Cymbopogen citratus* and *Mentha arvensis* (Manzoor *et al.*, 2012). In another study by Franca *et al.* (2012), it was seen that *E. citriodora* could reduce the number *of Zabrotes subfascial* adults attracted to *Phaseolus vulgaris* seeds, which, in the end, can also significantly reduce the number of viable eggs when compared to control. Insect repellent activity is an essential feature of biopesticides for pest control as they play a direct role in the reduction of laying eggs and hence the emergence of adults, making it challenging to establish pest populations.

3.5. Insecticidal activity

Several studies are showing that *E. citriodora* has an insecticidal activity in several types of insects such as *Lutzomyia longipalpis* (Maciel *et al.*, 2010), *Periplaneta americana* (Manzoor *et al.*, 2012), *Z. subfasciatus* (Franca *et al.*, 2012), *Anopheles gambia* (Bossou *et al.*, 2013), *Myzus persicae* (Costa *et al.*, 2015), *Frankliniella schultzei* (Costa *et al.*, 2015), *Tenebrio molitor* (Salazar *et al.*, 2015), *Tribolium castaneum* (Bossou *et al.*, 2015), *S. oryzae* (Tamgno *et al.*, 2019), *Aedes aegypti* (Manh *et al.*, 2020), *Ascia monuste* (Santos *et al.*, 2020), *Plutella xylostella* (Santos *et al.*, 2020), and *S. zeamais* (Ataide *et al.*, 2022),

E. citridora essential oil was recorded to cause 100% mortality of *Z. subfasciatus* (Franca *et al.*, 2012) and *A. gambiae* (Bossou *et al.*, 2013). The *E. citriodora* used on *A. gambiae* in the research came from Benin, which contains citronella, citronellol and isopulegol as its main compounds. The study showed that *E. citriodora* is also quite effective against resistant strains of *A. gambiae* compared to permethrin at the tested diagnostic dose (Bossou *et al.*, 2013). Another test conducted on the larvae of *A. aegypti* showed an LC50 value of 104.4 ppm. The *E. citriodora* essential oil used in this test contained 78.6% citronellal and 12.1% citronellol (Manh *et al.*, 2020).

In a study by Costa *et al.* (2015), *E. citriodora* essential oil can cause high mortality in *M. persicae* and low mortality in *F. schultzei.* It has an LC50 value of *M. persicae* by 0.40%, where the significant compounds of *E. citriodora* used in this study were citronellal (29.31 %) and geraniol (27.63%), β-citronellol (14.88 %) and δ-cadinene (6.32 %).

Another test carried out on *T. castaneum* showed that the most efficient was *E. citriodora* (82% mortality at 8%), compared to *Cymbopogon citratus* (18% at 8%), *Cymbopogon schoenanthus* (73% at 8%), and *Cymbopogon giganteus* (67% at 8%). The mortality obtained from the fumigation method was 75% for *E. citriodora.* This percentage was higher than the others, i.e., 71.7% for *C. schoenanthus*, 68.3% for *C. giganteus,* and 41.7% for *C. citratus* (Bossou *et al.*, 2015).

It impacts insect mortality, and *E. citriodora* essential oils can also cause sub-lethal effects. A study conducted by Ataide *et al.* (2022) showed that the emergence and survival in adults of *S. zeamais* were decreased due to the influence of *E. citriodora* (in this case, the percentage of citronellal as the significant compound was 83.95%).

In a test on *L. longipalpis*, *E. citriodora* was effective against the egg, larval, and adult phases, including inhibiting egg hatching. However, the egg phase was the most resistant to the Eucalyptus oils. This survival may also be due to the neurotoxic action of compounds that act only after the embryonic nervous system grows or because of the lower permeability of the egg surface at the start of embryogenesis (Maciel *et al*., 2010).

In another study on *Spodoptera frugiperda*, the percentage of larvae that reached pupae and emergence from the pupae decreased significantly affected by these substances from essential oils *E. citridora*. The rest even emerged with abnormalities or deformities. The average time to reach the mean weight of the adult stage relative to control larvae was also significantly delayed. The developmental and growth disruption happens during the pharate conditions following molting (apolysis) initiation but before molting (ecdysis) completion. This growth inhibition may be due to the inhibition of proteinase, ETH and another polyphenol oxidase (PPO).

Meanwhile, the tests conducted on *T. molitor* showed that *E. citriodora* could accelerate or shorten the timing of pupation and emergence. Even many of the pupae that reached adulthood eventually died (not emerged). From these two types of insect pests, it can be seen that the effect of *E. citriodora* can increase or decrease development time (Salazar *et al*., 2015).

Several studies suggest that *E. citriodora* may have an IGR (Insect Growth Regulator) effect. Insecticides with growth regulation properties can hurt insects by regulating or inhibiting pathways or biochemical processes that are important for the growth and development of insects (especially the process of metamorphosis or reproduction). Some insects exposed to these compounds can die due to abnormal development, prolonged exposure to external factors (susceptibility to natural enemies and environmental conditions), or cessation of insect development. Therefore, IGR does not necessarily cause death directly but can cause abnormalities that impair insect survival (Tunaz and Uygun, 2004).

In the research conducted by Santos *et al*. (2020), the compounds contained in *E. citriodora* were tested separately. The results showed that citronellal and citronellol were the most toxic compounds because they showed the lowest lethal doses for *A. monuste* and *P. xylostella*. At the same time, other compounds like α-pinene, (-)-isopulegol, trans-caryophyllene, and β-pinene presented low toxicity. However, when the test was carried out by fumigation, the results showed that citronellol had no insecticidal effect (0% mortality), while citronellal caused 82% mortality. Other compounds commonly found in eucalyptus, such as citronellyl acetate and piperine, can cause mortality of 2% to 100% (Bossou *et al*., 2015).

Other plants such as Geranium also have citronellol as a constituent compound. The main compounds that makeup geranium oil include: citronellol (38%), geraniol (16%), citronellyl formate (10.4%), and linalool (6.45%). In tests conducted on *Pediculus humanus capitis*, the results showed that when citronellol was removed, the highest decrease in toxicity occurred (DL50 from 2.2 to 10.9 μg/insects). Toxicity data proves that citronellol is the major contributor to geranium oil toxicity (Gallardo *et al*., 2012). Several studies show that citronellal and citronellol components are harmful in *E. citriodora* oil.

4. CONCLUSION

The above review shows that *E. citridora* essential oil has a broad spectrum of biological activity against weeds, fungi, bacteria, nematodes, and insects. It provides a simple and environmentally friendly alternative to pest control. Henceforth, further experiments are needed to evaluate the economic aspects and potential toxicology for non-target organisms. The activities under field conditions also need to be carried out further.

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