International Journal of Agricultural Technology 2024 Vol. 20(4):1379-1388
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Acaricidal activities of essential oil emulsions in controlling different mite pests

Doungnapa, T.[1], Pumnuan, J.[2*], Lakyat, A.[2] and Thipmanee, K.[2]

[1]Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani 12120, Thailand; [2]School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.

Doungnapa, T., Pumnuan, J., Lakyat, A. and Thipmanee, K. (2024). Acaricidal activities of essential oil emulsions in controlling different mite pests. International Journal of Agricultural Technology 20(4):1379-1388.

Abstract The result revealed the citronella grass emulsions had a highly toxic effect to the African red mite that presented LC_{50} and LC_{90} with 0.146 and 0.303%, respectively. While citronella grass emulsion at concentration 0.4% was able to control those 3 mite pest species in greenhouse or field conditions, when it presented the mite mortality with >80% compared with control group.

Keywords: Essential oil emulsion, African red mite, Orchid flat mite, Broad mite, Toxicity

Introduction

The mite pests are small living things that cannot be seen with the naked eye on the surface of leaves. The correct mite identification is also important because mites are not all pests. Many types of predatory mites can be found on the leaves these are beneficial as natural enemies of the plant mites (Mite and Spider Research Section, 2013; Jeppson *et al*., 1975). African red mite (*Eutetranychus africanus*) (Actinedida; Tetranychidae) was relatively the most important mite pest species that attacked the greatest of plants. Whereas, other mite pests as orchid flat mite (*Tenuipalpus pacificus*) and the broad mite (*Polyphagotarsonemus latus* (Banks)) especially destroy plants such as citrus, strawberry, apple, soybean, vegetables, peppers, cassava and a variety of ornamentals. During every growth stage of mites, the mites suck fluid from plant leaves. In cases of severe outbreaks, it may ultimately impact plant growth and yield. (Jeppson *et al*., 1975; Meyer, 1974; Meyer, 1987).

Nowadays, control management of these mites focuses mainly on chemical applications with novel modes of action and these are becoming increasingly important in modern agriculture as a component of integrated pest management and resistance management strategies. Kulpiyawat *et al*. (2002) informed the resistance of the African red mite to several insecticides such as amitraz, dicofol, bromopropylate and propakite. In certain northern

[*]**Corresponding Author**: Pumnuan, J.; **Email:** jarongsak.pu@kmitl.ac.th

regions, researchers discovered that every African red mite species showed resistance to all chemicals. Resorting to chemical control for mites escalates production expenses and poses environmental hazards. Although the combined applications of pesticides are a labor-saving shortcut method, an understanding and knowledge of pesticide compatibility is necessary to avoid problems arising from combinations of some pesticides and may cause unacceptable plant phytotoxicity of many insecticides. For another controls, Lo. (2002) This has stimulated increased interest in integrated pest management approaches. Biological control, in particular, may make pest suppression in this system more durable. In enclosed environments, it was simpler to establish natural enemies' populations, enabling more effective pest control without the detrimental effects of chemical pesticides compared to field situations. Insung and Pumnuan (2008) reported that the essential oil of citronella grass (*Cymbopogon nardus*) was extremely harmful to *Dermatophagoides pteronyssinus* after fumigation at 24 hrs with LD_{50} value at 0.935 µg/cm^3. When, Insung *et al*. (2008) showed that extract from *Piper retrofractum* could completely control the African red mite at 1% concentration. Besides, the essential oil of *Piper nigrum* presented an acaricidal efficacy on the spider mite, *Eutetranychus cendanai* with LC_{50} at 23.6 ml/l (Sornlek, 2001).

The study aimed to verify the toxicity property of essential oil emulsions from citronella grass (*Cymbopogon nardus* Rendle), lemon grass (*Cymbopogon citratus* (Dc.exNees)), clove (*Syzygium aromaticum* (L.) Merr. & L.M. Perry) and cinnamon (*Cinnamomum bejolghotha* (Buch-Ham.) Sweet) against the African red mite, the orchid flat mite and the broad mite.

Materials and methods

Mite culture and preparation

The African red mite: the African red mite (*Eutetranychus africanus* [Tucker]) was cultivated in the laboratory. Initially, they were raised on mulberry (*Morus alba* L.) leaves in which were placed on a cotton swab and soaked with water on a tray, serving as their food source. Thousands of African red mite adults were pet on mulberry leaves and kept at room temperature (25 ± 2 °C) and 85 ± 2% relative humidity (RH). Every 4 to 5 days, each leaf was replaced with a fresh one.

The orchid flat mite culture: The orchid flat mite (*Tenuipalpus pacificus* Baker.) adults were randomly collected from an orchid (*Dendrobium* Sonia 'Earsakul') from insecticide or acaricidal free orchard application in Nakorn Pathom province, Thailand

The broad mite culture: The broad mite (*Polyphagotarsonemus latus* Banks) reared on the seedling of chilli pepper in the insectary. The adults were randomly collected from a chilli pepper (*Capsicum* spp.) from insecticide or acaricidal free garden in Nakhon Ratchasima province, Thailand

Essential oil emulsion preparation

The essential oils obtained from citronella grass (*Cymbopogon nardus* Rendle), lemon grass (*Cymbopogon citratus*), cinnamon (*Cinnamomum bejolghota*), and clove (*Syzygium aromaticum*) were purchased from Thai-China Flavors and Fragrances Industry Co., Ltd., Thailand.

Citronella grass and lemon grass emulsions were prepared with essential oil: surfactant (Tween20): Co-surfactant (PEG400) ratios of 2:8:7 and 1:4:1, respectively. While cinnamon and clove emulsion were prepared with essential oil: surfactant (Tween60): Co-surfactant (PEG400) ratios of 2:9:5 and 2:9:2, respectively. All essential oil emulsions at 1.0% concentration in water were analyzed for their zeta potential and particle size was evaluated using a Nano Particle/Nano Plus Zeta Analyzer.

Bioassay

Toxicity properties under laboratory conditions

The African red mite: The effectiveness of 4 essential oil emulsions on the African red mite was primary estimated using the leaf dipping method. Mulberry leaves were cut into 2.7 cm diameter in circles and dipped for 1 minute in each of the essential oil emulsions at various concentrations and discharged to air-dry at room temperature (25 ± 2 °C, 85 ± 2% RH) for 15 minutes. About 10-15 adult female mites were screened under a microscope and introduced to the treated leaves. The mortality of mites was observed at 24 hours. In case of death mite occurred in control, was applied according to the actual death rate of Abbott's formula (Abbott, 1987) with the formula as follows:

$$\text{Mortality (\%)} = [T - C/100 - C]\times 100.$$

Where, T is test mortality (%) with essential oil emulsions, and C is control mortality (%) with comparison experiment.

The Orchid flat mite: The acaricidal property of selected essential oil emulsions formulas against the orchid flat mite (*T. pacificus*) was evaluated by direct spray method. The 3x4 inch orchid leaves were sprayed with selected essential oil emulsion at concentrations: 0.0% (water as control) and 0.4%, as well as pyridaben insecticide at (1X) by using a volume of 1.5 ml. It was left at room temperature to air-dry. Amount of 20-60 orchids flat mites were randomly collected from infested orchid leaves. The number of surviving orchid flat mites was observed 24 hours after treatment and compared to the control. The experiment was designed using a completely randomized design (CRD).

The Broad mite: The acaricidal property of selected essential oil emulsion formulas on the broad mite (*P. latus*) was evaluated by using also direct spray method. The bioassay was applied by spraying the selected essential oil emulsion on the chili leaves at concentrations: 0.0% (water as control), 0.4%, as well as amitraz insecticide at (1X) for 1.5 ml. It was left at room temperature to air-dry. Amount of 10 broad mite adults were placed on the chili leaves. The mortalities of broad mite were observed at 24 hours after treatment. The experiment was designed using a completely randomized design (CRD).

Toxicity properties under greenhouse conditions

Acaricidal test in greenhouse conditions of essential oil emulsion formulas of citronella grass the most effective essential oils on African red mite was performed by direct spray method. At concentrations 0.4% compared with the insecticide (Amitraz 1X), at recommendation rates were examined. The experiment was performed on mulberry seedling planted 2 months in a single pot (1 plant per pot), About 100 adults of African red mites were released and randomly counted was made after 3 days as number of mites/trees before and after spraying. The surviving African red mites were counted on branches and leaves and compared with the control. The experiments were conducted based on RCBD and five replications.

Toxicity properties under field conditions

Following the laboratory and greenhouse conditions studies, effective of selected essential oil emulsion formula was evaluated in field conditions. In this assay, the direct spray was applied to organic durian orchard (in Nakhon Ratchasima province) which was infested by the African red mite. The orchard was applied with selected essential oil emulsion at the 0.4% concentration and compared with insecticide (Amitraz) at the recommendation rate. The African red mites were randomly collected from durian leaves before and after spraying. The surviving African red mites were counted on durian leaves, compared with the control. The experiment was conducted on RCBD and 5 replications.

Statistical analysis

In this study, Abbott's formula was applied to achieve the mite actual death rate. The experiment used in the study was a completely randomized design (CRD) with 5 replicates per treatment. The data were analyzed using ANOVA, and the differences amid various treatments were tested by Duncan's multiple range test (DMRT) on 95% confidence level ($P<0.05$) in SAS program. For the experiment in the greenhouse conditions, in the RCBD (randomized completely block design) experiment was planned.

Besides, the acaricide toxicity in term of LC_{50} and LC_{90} was calculated using the probit analysis in SPSS.

Results

The emulsions of four essential oils against the African mite were then made to be essential oil emulsions by surfactants and co-surfactants at reasonable various ratios. It was found that more use of surfactants than co-surfactants caused the better stability of emulsions. If using amount of surfactant less than co-surfactant resulted in unsteadiness emulsions. The particle size analysis was done by using dynamic light scattering technique (DLS), obtained result informed that the particle size of the citronella grass essential oils, lemon grass, clove and cinnamon ranged from 11.80-34.57 nm diameter, with polydispersity index of 0.04-0.19 and zeta potential at (-4.39) to (-7.92) mV (Table 1).

Table 1. Particle sizes of essential oil emulsions

Essential oil emulsions	Essential oil : Surfactant : Co-Surfactant ratio	Parameter	Particle size and zeta potential assessment
Citronella grass emulsion	Citronella grass oil : Tween20 : PEG400 = 2 : 8 : 7	Diameter (nm)	11.80±0.10
		Polydispersity Index	0.04±0.00
		Zeta Potential (mV)	-6.95±1.02
Lemon grass emulsion	Lemon grass oil : Tween20 : PEG400 = 2 : 9 : 5	Diameter	34.57±2.74
		Polydispersity Index	0.19±0.02
		Zeta Potential	-4.39±0.47
Cinnamon emulsion	Cinnamon oil : Tween60 : PEG400 = 2 : 8 : 7	Diameter	18.70±0.20
		Polydispersity Index	0.15±0.02
		Zeta Potential	-6.23±2.33
Clove emulsion	Clove oil : Tween60 : PEG400 = 2 : 8 : 7	Diameter	16.63±0.12
		Polydispersity Index	0.14±0.00
		Zeta Potential	-7.92±0.74

The toxicity property test of essential oil emulsions of medicinal plant to African red mite (*E. Africanus*) revealed that 4 essential oils emulsions at higher concentration caused the higher percentage of African red mite mortality. The essential oil emulsions of citronella grass were the most effective in killing the mite. However, the LC_{50} and LC_{90} of citronella grass gave the best values at 0.146 and 0.303, respectively. Followed by essential oil emulsions of lemon grass, cinnamon and clove, with their LC_{50} and LC_{90} values were 0.427, 0.924, 0.440, 0.792, 0.502 and 0.931%, respectively (Table 2). The obtained result demonstrated that normally various compounds existing in essential oil could labor together better than a chemical compound alone as well as there was some synergistic effect according to mixing between the precursor and surfactant.

Table 2. LC_{50} and LC_{90} of different essential oil emulsions against the African red mite by leaf dipping method under laboratory conditions

Essential oil emulsions	Regression equation	Chi-square	LC_{50} (Lower–Upper)	LC_{90} (Lower–Upper)	SE	P
Citronella grass emulsion	Y= 8.176x – 1.193	31.963	0.146 (-0.02–0.27)	0.303 (0.20–0.77)	0.785	0.000^{**}
Lemongrass emulsion	Y= 2.580x – 1.103	31.980	0.427 (0.17–0.62)	0.924 (0.70–1.61)	0.193	0.000^{**}
Cinnamon emulsion	Y= 3.645x – 1.605	14.352	0.440 (0.33–0.53)	0.792 (0.67–1.00)	0.244	0.000^{**}
Clove emulsion	Y= 2.983x – 1.497	19.083	0.502 (0.36–0.63)	0.931 (0.76–1.27)	0.208	0.000^{**}

** Represented significant difference at $P < 0.01$, * represented significant difference at $P < 0.05$, ^{ns} nonsignificant difference.

The effectiveness of the essential oil emulsions formula of citronella grass at selected concentrations of 0.4% was tested against the orchid flat mite and compared with the insecticide (Pyridaben), as well as tested against the broad mite and compared with the insecticide (Amitraz) at recommendation rate by direct spray method. The result demonstrated that essential oil emulsions of citronella grass showed highly toxic effect on the orchid flat mite and broad mite that only 0.4% concentration demonstrated 100% and 73.5% mite mortality, respectively. Whereas insecticide (Pyridaben and Amitraz) was not different from the control (Surfactant) (Table 3).

Table 3. Percentages mortality of orchid flat mite (*Tenuipalpus pacificus* (Baker)) and the broad mite (*Polyphagotarsonemus latus* (Banks)) caused by 0.4% concentrations of citronella grass essential oil emulsion by direct spray method under laboratory conditions

Essential oil emulsions	%Mortality	
	Orchid flat mite	Broad mite
Surfactant	0.00b[1]	8.40b
Citronella grass 0.4%	100.00a	73.5a
Amitraz	-	7.50b
Pyridaben	0.00b	-

[1] Means in column with the same stored condition followed by the same super script are not significantly different at $p < 0.05$ as determined by DMRT.

The experiment of using 0.4% concentration of citronella grass essential oil emulsion to suppress the African red mite and compared to chemical acaricide, Amitraz at the recommendation rate was performed by direct spray method, obtained result showed that the citronella grass emulsion presented the maximum control percentage of 100% under laboratory and greenhouse. And control percentage of African red mites was only 82.31%, whereas Amitraz acaricide exhibited only 55.09% under field conditions. and the control group (Surfactant) could a little bit decrease the mite 10.40-20.76% with significant differences. (Figure 4).

Table 4. Percentages mortality of African red mite (*Eutetranychus africanus* (Tucker)) caused by 0.4% concentrations of citronella grass essential oil emulsion by direct spray method under laboratory, greenhouse, and field conditions

Essential oil emulsions	%Mortality		
	Laboratory conditions	Greenhouse conditions	Field conditions
Surfactant	10.40b[1]	14.97b	20.76c
Citronella grass 0.4%	100.00a	100.00a	82.31a
Amitraz	100.00a	100.00a	55.09b

[1] Means in column with the same stored condition followed by the same super script are not significantly different at $p < 0.05$ as determined by DMRT.

Discussion

The 4 most effective essential oils against the African mite were then formulated into emulsions using of various appropriate ratios surfactants and co-surfactants. The application of more volume of surfactants than co-surfactants caused the better stability of emulsions. Normally, using less amount of surfactant than co-surfactant imparts unsteadiness emulsions. The selected surfactants have to be able to decrease the interfacial tension to a very small dosage to encourage the dispersal processing in the emulsion provision to obtain the desirous emulsion type (i.e., water/oil, oil/water, or bicontinuous) (McClements, 2011; Solans and Solé, 2012; Komaiko and McClements, 2015; Kwon *et al.*, 2015; Jintapattanakit, 2018). The polydispersity index indicates the quality of size distribution (Danaei *et al.*, 2018). Zeta potential values above 30 mV suggested physical stability (Marsalek, 2014). Unfortunately, poor zeta potential values were detected in this study. It has been clearly observed that emulsions containing small particles of plant essential oils performed better than emulsions containing larger particles. In addition, the use of co-surfactants was a very important technique for preparing essential oil emulsions, as their mixing may have a promotional effect on the mites. Higher concentrations of all essential oil emulsions resulted in higher mortality rates of the African red mite. Citronella grass emulsions on 0.4% could kill up to 100% of the mites. There were very few reports related to the effectiveness of citronella grass essential oils in controlling mite pests. Pumnuan and Insung (2016) presented that lemon grass and citronella grass essential oils to the mushroom mite (*Dolichocybe indica*) using the fumigant method at 3 µg/L air incur 100% and 82.5% mortality, respectively. Moreover, Saad *et al.* (2017) reported that citronella essential oil was composed of widely insecticidal properties such as contact toxicity, repellency and inhibition of egg laying against *Bemisia tabaci*. It also caused high mortality at the concentration of 6.66 lL/L air. Mwandila *et al.* (2013) showed that syringa (*Melia azedarach*) seed extracts (SSE) exhibited acaricidal properties against the eggs, nymphs and adults of red spider mite (*Tetranychus* spp.). Unfortunately, any reports about the intervention of surfactants to mite egg-laid were not found. At concentration of 0.4% of citronella grass was used to test to the orchid flat mite and the broad mite by direct spray method. The obtained result explained that the citronella grass was extremely effective in controlling the orchid flat mite and the broad mite. Choi *et al*. (2004) Tested the toxicity of 53 plant essential oils to eggs and adults of *T. urticae* including adults of *Phytoseiulus persimilis* were found that demonstrated citronella java, lemon, eucalyptus and peppermint oils, and was a higher mortality rate of 90% among adults *T. urticae*. Miresmaili *et al*. (2006) evaluated the toxicity of *Rosemarinus officinalis* L. essential oil and a combination of main components with *T. urticae* on different host plants. It was found that while some constituents were responsible for most of the toxicity, others were relatively inactive. According to this study, the essential oil emulsions tend to be used to control the mites in field conditions. When Mead (2012) informed that lemon grass oil was found to be more toxic to *T. urticae* adults using the spray method than the leaf dipping method. The percentage of egg hatchability of *T. urticae* was significantly reduced than that of the control. Increasing studies on the chemical interactions of plant insects over the past few decades revealed the potential of using botanical insecticides in the form of secondary plant metabolites, or allelochemicals as pesticides. This is interested in natural pesticides derived from leaves or seeds offers an alternative to synthetic insecticides in pest management programs (Whitten, 1992; Martinez and van Emden, 2001).

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(Received: 14 October 2023, Revised: 7 April 2024, Accepted: 20 May 2024)