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SCREENING OF FIVE LAMIACEAE ESSENTIAL OILS AS REPELLENTS FOR SWEET POTATO WEEVIL, *CYLAS FORMICARIUS (F.)* (COLEOPTERA: BRENTIDAE)

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ABSTRACT
In this research, the repellent activity of five Lamiaceae essential oils, *Agastache rugosa*, *Elsholtzia blanda*, *Elsholtzia ciliata*, *Elsholtzia penduliflora*, and *Plectranthus ovatus*, was evaluated against sweet potato weevil, *Cylas formicarius* using a two-choice test between the essential oil-treated filter paper and the control. *E. blanda* and *A. rugosa* essential oils have some sweet potato weevil attractant properties at low dose (<47.16 nl/cm^2),while N,N-diethyl-3-methylbenzamide (DEET), *P. ovatus*, *E. penduliflora*, and *E.ciliata* essential oils have repellent properties at doses ranging from 15.72 nl/cm^2 to 196.49 nl/cm^2. The effect of type of essential oil and their dose were interactively associated with repellent efficacy. There was a increase in repellent efficacy as the dose increased for all essential oils. The repellent activities of *P. ovatus* essential oil and *E. penduliflora* were higher than the others, and the repellent effects of *E. ciliata* essential oil and DEET were more dose-dependent than others, indicating that at low dose, *P. ovatus* and *E. penduliflora* essential oils have stronger repellent efficacy, but at higher dose DEET and *E. ciliata* have greater effects. Our findings clearly demonstrate that *P. ovatus*, *E. penduliflora*, and *E. ciliata* essential oils are candidate materials for future investigation as repellent compounds against sweet potato weevil control.

Contribution/Originality: This study uses new estimation methodology to characterize the role of types of essential oils and their dose in the response of insects to repellents.

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1. INTRODUCTION
Sweet potato weevil, *Cylas formicarius* (Fabricius) (SPW), is the most important insect pest infesting sweet potato, both in the field and in storage (Sutherland, 1986). Their impact on sweet potato crops results in millions of dollars of losses annually around the world (Jackson et al., 2005). Vietnam, among the ten largest sweet potato-producing

countries in the world (1994–2019) (FAOSTAT, 2021), recorded losses of up to 30-40% (Dinh, Khang, & Thew, 1995). SPW larvae bore into the vines and tubers of sweet potatoes (Sutherland, 1986); infested sweet potato tubers then produce certain terpenoids, resulting in infested tubers becoming unsuitable for consumption by either humans or livestock (Sato, Uritani, & Saito, 1982). Thus, even a low level of infestation by SPW causes considerable economic losses. This pest is mainly controlled by insecticides and pheromone trapping. Chemical insecticides are understood to give inadequate control (Sutherland, 1986) because the larvae develop inside the vines and tubers. Moreover, sex pheromones attract only males, leaving females to continue to cause damage (Proshold, Gonzalez, Asencio, & Heath, 1986). Therefore, the efforts to control SPW still face various challenges.

Insect olfaction refers to the function of chemical receptors that enable insects to detect and identify volatile compounds for host selection, defense and escape from predators, shelter selection, mating and reproduction, and general orientation (Carraher et al., 2015). Thus, it is the most important sense for insects. Insects are capable of detecting and differentiating between thousands of volatile compounds, both sensitively and selectively (Carraher et al., 2015; Syed, 2015). Therefore, chemosensory-based tactics are especially important for integration of insect pest management. The strategy for repellents use is mainly to stop pests from finding a valuable resource. Useful repellents can be derived from natural sources, such as plants (e.g. essential oils), or they may synthesize, as in the case for most insecticides (Curtis, Lines, Baolin, & Renz, 1990; Norris, 1990). Most efforts to apply volatile repellents have protected humans from insect bites, particularly from insects such as mosquitoes and blackflies that are vectors of diseases (Schreck, 1977). Among these chemicals, the best known is DEET, which is used to repel a broad range of insects (Rutledge, Sofield, & Moussa, 1978). Repellents protecting crops, excepting those with general insecticidal activity, have received little attention (Renwick, 2018). We therefore suggest that the use of repellent plants as push-plants (push–pull strategies) or compounds (easy to synthesize, easy to apply or deliver, inexpensive, low-toxicity…) with repellent activity against SPW may contribute to the Integrated Pest Management strategy for SPW in the field, as well as in storage.

Plants members of the Lamiaceae, especially *Ocimum* spp., are used as traditional mosquito repellents globally (Ntonifor, Ngufor, Kimbi, & Oben, 2006; Seyoum et al., 2002). Numerous studies have shown that essential oils and their constituents from Lamiaceae have high repellent activity against insect pests (Maia & Moore, 2011; Nerio, Olivero-Verbel, & Stashenko, 2010). In addition, recent research has shown that mulching with fresh material from Lamiaceae (basil (*Ocimum basilicum* L.), lime basil (*Ocimum americanum* L.) and spearmint (*Mentha spicata* L.)) reduced the number of weevil feeding holes on sweet potato storage root fragments, and the numbers of weevil (Rehman, Liu, Johnson, Dada, & Gurr, 2019). Another study indicated that the use of Lamiaceae (oregano (*Origanum vulgare* L.), basil (*Ocimum basilicum* L.) and mint lime (*Mentha* × *piperita* L.)) as barrier plants reduced the passage of *C. formicarius* and the number of oviposition holes in sweet potato storage roots (Dada, Liu, Johnson, Rehman, & Gurr, 2020). Therefore, Lamiaceae essential oils are potential candidates as repellents against SPW.

This study aims to assess the repellent activity of Lamiaceae essential oils as a basis to invest repellent compounds of essential oils as candidates for synthesis and use as active repellent ingredients in the field, as well as in storage. Furthermore, the stems and leaves of the plants whose essential oils have repellent activity can be used to mulch sweet potato in the field or to cover piles of sweet potato in storage.

2. MATERIAL AND METHODS

2.1. Plant and Essential Oils

The aboveground parts of five Lamiaceae plants were collected (during their flowering periods) in Son La and Lao Cai provinces of the Northwest region of Vietnam (Table 1). After sample collection, the plants were cut into small pieces of 5–7 cm in length then spread in thin layers in a well-ventilated room for about 12 hours to reduce the water in the plants before essential oil extraction.

Essential oils of five Lamiaceae species were extracted by steam distillation using a stainless steel distillation apparatus. Three liters of filtered water and 1 kg of plant materials were used to collect 1.5 l of condensed water. Subsequently, the essential oils were separated using a pipette. The essential oils collected over water were dried over sodium sulfate and preserved in a refrigerator (4°C) for subsequent experiments.

A commercial repellent, DEET, was purchased from Sigma and used as a positive control.

Table-1. Names and sources of five Lamiaceae essential oils used in this study.

Scientific name	Common name	Collection time	Collection location	Latitude and longitude	Elevation
Agastache rugosa (Fisch. & C.A.Mey.) Kuntze	Korean mint	June, 2016	Son La province	21° 16’ 57.9” N 103° 55’ 28.3” E	795 m
Elsholtzia blanda (Benth.) Benth.	-	November, 2016	Lao Cai province	22° 21’ 20.7” N 103° 51’ 36.5” E	1351 m
Elsholtzia ciliata (Thunb.) Hyl.	Vietnamese balm	November, 2016	Son La province	21° 18’ 02.1’ ’N 103° 56’ 00.6” E	666 m
Elsholtzia penduliflora W.W.Sm.	-	November, 2016	Lao Cai province	22° 21’ 14.6” N 103° 51’ 48.1” E	1296 m
Plectranthus ovatus Benth.	Clove basil	August, 2016	Son La province	21° 17’ 27.2” N 103° 55’ 53.2” E	804 m

2.2. Insect Culture

The *Cylas formicarius* (SPW) specimens used in this study were originally collected in Tay Hung village, Muoi Noi Ward, Thuan Chau District, Son La province in 2016, and were maintained for more than 15 generations on sweet potato root. The SPW adults used for testing were generated by 200 pairs of sexually mature SPW, which were placed in a plastic cage containing 2 kg of sweet potato for 2 days. The plastic cage is 28 cm in diameter and 25 cm in height and was covered by a mosquito net. After this period, the adults were removed and the cages containing the sweet potatoes were incubated at 27 ± 1ºC and 75 ± 5% RH under a 14:10 light:dark photoperiod. When the adults emerged from the tubers, male and female weevils were separated daily. The adults emerging from the tubers after 2–6 days were used for experimentation.

2.3. Repellent Bioassay

The repellence test used was adopted from the method of Liu and Ho (1999), with some modifications. Petri dishes (9 cm in diameter) were used to confine SPW during the experiment. DEET and essential oils were diluted in n-hexane to different concentrations (15.72, 47.16, 78.63 and 196.49 nl cm^{-2}), and pure n-hexane was used as the control. Filter paper (9 cm in diameter) was cut in half and 500 µl of each concentration was applied separately to one half of the filter paper as uniformly as possible, with a micropipette. The other half (control) was treated with 500 µl of absolute n-hexane. Both the treatedand control halves were then air-dried to evaporate the solvent completely. After drying, the treated and control halves were reassembled with solid glue on a Petri dish. Twenty adults (10 males and 10 females) were released in the center of each filter paper disc. Vaseline was applied to the inner vertical side of the Petri dishes to prevent the SP from climbing on to the sides and the lid of the dishes. A cover was then placed over the dish. Because adult walking activity is greatest under darkness (Shimizu & Moriya, 1996), the testing period was between 6 pm and 11 pm at 26 ± 2 °C and 75 ± 10% RH. Each treatment was repeated three times. Counts of insects located on each strip were made after 2 and 4 hours. The repellent effect was measured by comparing the counts of insects on the control and treatment strips – more insects on the control strip indicated the repellent effect of the treatment strip.

Percentage repellence (PR) for a given exposure time was calculated using the formula: PR = $[(N_c - N_t)/(N_c + N_t)]$, where N_c and N_t are the number of insects presented in the control and treated half, respectively.

2.4. Statistical Analysis

Repellence data was processed with R (R Development Core Team, 2020) using a logistic regression model with two factors (essential oil and dose) and interactions:

$$p_{ij} = \frac{e^{\alpha+\beta*O_i+\gamma*D_j+\lambda*O_i*D_j}}{1+e^{\alpha+\beta*O_i+\gamma*D_j+\lambda*O_i*D_j}}$$

in which p_{ij} is the predicted value of the proportion of SPW choosing the treated area/total essential oil i at dose j, O_i is essential oil i, D is dose, α is the intercept, β is the parameter estimate of the effect of essential oil, γ is the parameter estimate of the effect of dose, and λ is parameter estimate of the effect of interaction between oil and dose.

3. RESULTS AND DISCUSSION

The results of repellence essays for five Lamiaceae essential oils and DEET against SPW are presented in Table 2. The data show that at the lower tested concentrations (15.72 nl/cm² and 47.16 nl/cm²), there was no repellence of *E. blanda* and *A. rugosa* essential oils, but some insect attractant properties were observed (Table 2 and Figure 1). DEET, *P. ovatus*, *E. penduliflora*, and *E.ciliata* essential oils possessed repellent properties at all tested concentrations (Table 2 and Figure 1).

Table-2. Percentage of repellence after two exposure times for five Lamiaceae essential oils and DEET against SPW.

Essential oil	RE (%) (mean ± SE)			
	15.72 nl/cm²	**47.16 nl/cm²**	**78.63 nl/cm²**	**196.49 nl/cm²**
	2 h			
DEET	26.7 ± 12.0	70.0 ± 25.2	70.0 ± 15.3	100.0 ± 0.0
E. penduliflora	43.3 ± 6.7	56.7 ± 13.3	56.7 ± 8.8	70.0 ± 5.8
P. ovatus	63.3 ± 18.6	93.3 ± 3.3	76.7 ± 13.3	83.3 ± 8.8
E. ciliata	26.7 ± 6.7	80.0 ± 5.8	56.7 ± 12.0	83.3 ± 6.7
E. blanda	-6.7 ± 21.9	-23.3 ± 14.5	66.7 ± 18.6	30.0 ± 26.5
A. rugosa	3.3 ± 12.0	-13.3 ± 8.8	-16.7 ± 3.3	36.7 ± 17.6
	4 h			
DEET	0.0 ± 10.0	46.7 ± 18.6	76.7 ± 14.5	96.7 ± 3.3
E. penduliflora	20.0 ± 30.0	83.3 ± 8.8	63.3 ± 8.8	70.0 ± 10.0
P. ovatus	40.0 ± 20.0	66.7 ± 12.0	83.3 ± 6.7	86.7 ± 8.8
E. ciliata	16.7 ± 3.3	60.0 ± 5.8	73.3 ± 6.7	93.3 ± 6.7
E. blanda	0.0 ± 11.5	13.3 ± 12.0	73.3 ± 17.6	43.3 ± 8.8
A. rugosa	13.3 ± 17.6	-16.7 ± 8.8	20.0 ± 5.8	30.0 ± 0.0

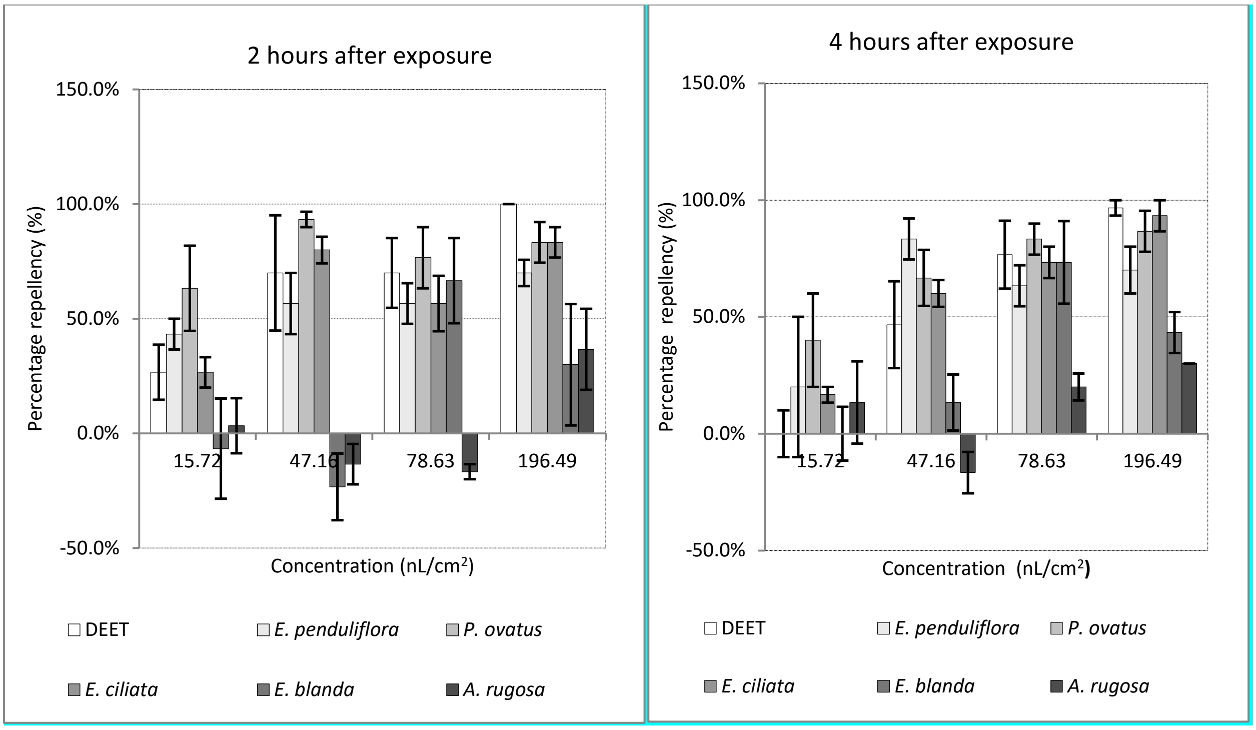

Figure-1. Repellent efficacy of five Lamiace essential oils and DEET at different dosages against SPW at 2 and 4 hours after exposure.

Table-3. Summary of logistic regression analysis of the effect of the interaction of essential oils and dose on the proportion of SPW choosing the treated area.

Factors (variables)	Comparison unit	Analysis of deviance			Parameter estimate		
		Deviance	df	*P*	Estimate	SE	*P*
		2 h					
Intercept (α)					-0.2222	0.3096	0.473
Type of essential oil (β)	DEET	141.4	5	<0.001			
E. penduliflora					-0.7382	0.3933	0.061
A. rugosa					0.5820	0.3727	0.118
P. ovatus					-1.6751	0.4489	<0.001
E. ciliata					-0.5716	0.3999	0.153
E. blanda					0.2906	0.3730	0.436
Dose (γ)	nl/cm²	41.9	1	<0.001	-0.0244	0.0061	<0.001
Essential oil: dose (λ)	DEET: dose	17.8	5	<0.01			
E. penduliflora: dose					0.0203	0.0066	<0.01
A. rugosa: dose					0.0195	0.0064	<0.01
P. ovatus: dose					0.0212	0.0070	<0.01
E. ciliata: dose					0.0155	0.0069	<0.05
E. blanda: dose					0.0195	0.0065	<0.001
Residual		141.4	60	<0.001			
		4 h					
Intercept (α)					0.3302	0.297	0.265
Type of essential oil (β)	DEET	70.6	5	<0.001			
E. penduliflora					-1.2670	0.3858	<0.01
A. rugosa					-0.2930	0.3613	0.417
P. ovatus					-1.2812	0.4054	<0.01
E. ciliata					-0.5754	0.4044	0.155
E. blanda					-0.6021	0.3659	0.100
Dose (γ)	nl/cm²	67.8	1	<0.001	-0.0273	0.0058	<0.001
Essential oil: dose (λ)	DEET: dose	34.9	5	<0.001			
E. penduliflora: dose					0.0218	0.0064	<0.001
A. rugosa: dose					0.0240	0.0061	<0.001
P. ovatus: dose					0.0163	0.0069	<0.05
E. ciliata: dose					0.0085	0.0074	0.253
E. blanda: dose					0.0223	0.0062	<0.001
Residual		121.4	60	<0.001			

The repellent efficacy of DEET and five Lamiaceae essential oils was dependent not only on the type of essential oil but also the dose. Our results indicated significant differences in the proportion of SPWs choosing the treated area, as a function of the type of essential oil (compared with the effect of DEET) (deviance = 141.4, df = 5, $P < 0.001$), the effect of dose (deviance = 41.9, df = 1, $P < 0.001$), and the effect of interaction between the type of essential oil and dose (compared with the effect of interaction between DEET and dose) (deviance = 17.8, df = 5, $P < 0.01$) (Table 3).

There was a clear decline in the proportion of SPWs, which made the choice of choosing the treated area as the dose increased for all essential oils (Figure 2).

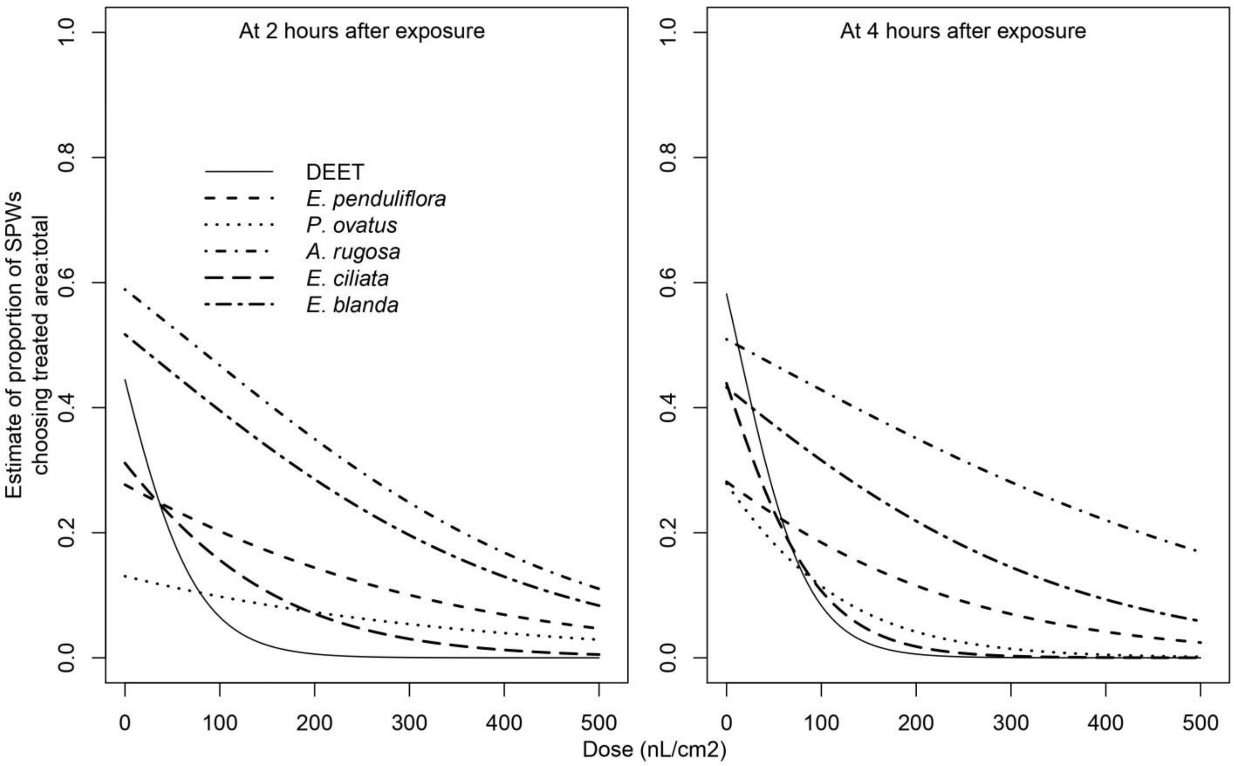

Figure-2. Logistic regression curves of proportion of SPW choosing the treated area/total with DEET, five Lamiaceae essential oils and dose.

Compared with the effect of DEET, only *P. ovatus* essential oil showed significantly higher repellent activity ($\beta_{P.ovatus}$= -1.6751, P<0.001) (Table 3). At low dose, *P. ovatus* essential oil exhibited the highest repellent activity. The repellent efficacy for SPW exposure to concentration 15.72 nl/cm^2, in order of effectiveness, was *P. ovatus* (63.3%) > *E. penduliflora* (43,3%) > *E. ciliata* = DEET (26.7%) > *A. rugosa* (3.3%) > *E. blanda* (-6.7%) (Table 2).

The effect of the interaction between DEET and dose was significantly different to the effect of that between essential oils and dose ($\lambda_{E. penduliflora}$= 0.0203, P<0.01; $\lambda_{A. rugosa}$ = 0.0195, P<0.01; $\lambda_{P. ovatus}$ = 0.0212, P<0.01; $\lambda_{E. ciliata}$ = 0.0155, P<0.05, $\lambda_{E. blanda}$ = 0.0195, P<0.001) (Table 3). This indicated that the effects of DEET were more dose-dependent than the others. Therefore, at higher dose, the repellent effect of DEET increased more than that of essential oils. At concentrations of 47.16 and 78.63 nl/cm^2, the order of efficacy, respectively, was *P.ovatus* (93.3%) > *E. ciliata* (80.0%) > DEET (70%) > *E. penduliflora* (56.7%) > *A. rugosa* (-13.3%) > *E. blanda* (-23.3%), and *P. ovatus* (76.7%) > DEET (70.0%) > *E. blanda* (66.7%) > *E. penduliflora* = *E. ciliata* (56.7%) > *A. rugosa* (-16.7%) (Table 2). At the highest tested dose (196.49 nl/cm^2), the order changed to DEET (100%) > *P.ovatus* = *E. ciliata* (83.3%) > *E. penduliflora* (70%) > *A. rugosa (36.7%)* > *E. blanda* (30.0%) (Table 2).

The results after 4 and 2 h of exposure were similar, except that both *P. ovatus* and *E. penduliflora* essential oils showed stronger repellent activity than DEET and the repellent efficacy of *E. ciliata* was dose-dependent equivalent to DEET and higher than the others (Table 3). At dose of 15.72 nl/cm^2, the repellent efficacy was *P. ovatus* (40.0%) > *E. penduliflora* (20.0%) > *E. ciliata* (16.7%) > *A.rugosa* (13.3%) > *E. blanda* = DEET (0%) in descending order, and these effects were *E. penduliflora* (83.3%) > *P. ovatus* (66.7%) > *E. ciliata* (60%) > DEET (46.7%) > *E. blanda* (13.3 %) > *A. rugosa* (-16.7%) at dose of 47.16 nl/cm^2 (Table 2). At the tested dose of 78.63 nl/cm^2 the order of repellent effect was *P. ovatus* (83.3%) > DEET (76.7%) > *E. ciliata* = *E. blanda* (73.3%) > *E. penduliflora* (63.3%) > *A. rugosa* (20%). Conversely, at the highest assayed dose (196.49 nl/cm^2), the efficacy was DEET (96.7%) > *E. ciliata* (93.3%) > *P. ovatus* (86.7%)> *E. penduliflora* (70%) > *E. blanda* (43.3%) > *A. rugosa* (30%) (Table 3).

It is clear that at the low dose, *P. ovatus* and *E. penduliflora* essential oils have stronger repellent efficacy but, at the high dose, DEET and *E. ciliata* have greater effects.

Many essential oils and their constituents were evaluated for repellence against insects (Maia & Moore, 2011; Nerio et al., 2010). However, only a few reports are available on bioactivity to the SPW, mainly regarding insecticidal activity (Facey, Porter, Reese, & Williams, 2005; McNeil, Porter, & Williams, 2012). In this study, for the first time, the repellent efficacy of five Lamiaceae essential oils against SPW was evaluated. Based on our results, we characterized the repellent properties of five Lamiaceae essential oils into three groups. Group 1 included *P. ovatus* and *E. penduliflora*, whose essential oils have higher repellent bioactivity than commercial DEET, but their efficacy was less dose-dependent than DEET. Group 2 included DEET and *E. ciliata*, which were characterized similarly DEET. Group 3 included *A. rugosa* and *E. blanda*, whose essential oils have repellent bioactivity equivalent to DEET, and their efficacy was less dose-dependent than DEET. These findings suggested that *P.ovatus*, *E. penduliflora*, and *E. ciliata* essential oils may contain effective repellent compounds with different properties, and need further investigation.

The strong repellent effects of *P. ovatus* essential oil against adult *Tuta absolut* (Essoung, Tadjong, Chhabra, Mohamed, & Hassanali, 2020), *Sitophilus oryzae*, *Tribolium castaneum*, *Ryzopertha dominica*, *Callosobruchus chinensis* (Ogendo et al., 2008), *Aedes aegypti* (Keziah, Nukenine, Danga, Younoussa, & Esimone, 2015), and *E. ciliata* essential oil against *Blattella germanica* (Huang, Zhang, Ren, Dong, & Wu, 2020) have been noted previously. These findings, considered together, suggest that the essential oils derived from Lamiaceae, especially *P. ovatus* and *E. ciliata*, show potential for the investigation of new and safe compounds to repel SPW.

P. ovatus, *E. penduliflora* and *P. ciliata* essential oils have high repellent activity oagainst SPW adults under laboratory conditions, and have provided information that may eventually result in implementation of an alternative management strategy to deal with SPW in the field. This may involve a "push–pull strategy" in which pests are repelled from the main crop using repellent plants (push) and then lured to the trap crop (pull) where they are concentrated, facilitating their elimination (Cook, Khan, & Pickett, 2007). This strategy may be easy to apply because all three Lamiaceae plants are inexpensive and easy to grow. In the future, the effectiveness of using Lamiaceae in push–pull strategies for SPW control in the field must be assessed.

In this study, we used a Petri dish bioassay to evaluate the repellent activity of essential oils. This method allows rapid screening of the repellent effect of essential oils with regular timing. However, in some cases, it can give an aberrant response. In Petri dish bioassay, SPWs walk on treated paper; at the same time, active chemicals could have toxic effects by contact, in addition to their repellent action.

4. CONCLUSION

In conclusion, our results indicated that *E. blanda* and *A. rugosa* essential oils have some SPW attractant properties at low dosage (<47.16 nl/cm^2), and DEET, *P. ovatus*, *E. penduliflora*, and *E.ciliata* essential oils possess repellent properties at a dosage range of 15.72–196.49 nl/cm^2. There was increased repellent efficacy as the dose increased for all essential oils. The repellent activities of *P. ovatus* essential oil and *E. penduliflora* were higher than the others tested, and the repellent effects of *E. ciliata* essential oil and DEET were more dose-dependent than the others. At low dose, *P. ovatus* and *E. penduliflora* essential oils have higher repellent activity against SPW than DEET. In contrast, at high dose, DEET and *E. ciliata* essential oils have a greater effect. *P.ovatus*, *E. penduliflora*, and *E. ciliata* essential oils are candidate materials for future investigation of repellent compounds for SPW control.

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