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Fumigant and repellent potentials of essential oil from carrot (*Daucus carota* L.) seeds against *Sitophilus zeamais* Motschulsky

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Abstract

The maize weevil, *Sitophilus zeamais* Motschulsky, is the most destructive insect pest of whole grains, both for breeder seed and preserved consumption. Fumigant toxicity and repellent effects of essential oil from *Daucus carota* seeds against adult *S. zeamais* were evaluated. All bioassays were vapor-phase tested. Results showed that essential oil from *D. carota* seeds had fumigation toxicity to adult *S. zeamais* at 24, 48 and 72 h with the median lethal concentration (LC_{50}) values of 26.27, 19.60 and 13.27 µL/L air, respectively. Efficiency of 100% mortality of adult *S. zeamais* with 64 µL/L air after treatment at 72 h was significantly different ($p < 0.01$). Essential oil of *D. carota* seeds had no repellent effect on adult *S. zeamais*, with an attractive effect of less than 50% repellent efficiency. Essential oil from *D. carota* seeds was highly toxic to adult *S. zeamais* by fumigation and showed possible use as an alternative stored grain protection against insect pests.

Keywords: Insecticide, Toxicity, Plant products, *Daucus carota*, *Sitophilus zeamais*, Essential oil

1. Introduction

Insect pests cause quantitative and qualitative losses to stored grain products for farmers [1]. *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is a serious pest of stored grain in Thailand [2] with wide distribution in hot and humid conditions [3, 4]. Damage caused by *S. zeamais* on stored rice grain is extremely high in tropical and subtropical regions [5], decreasing the weight of agricultural products and, consequently, market value, seed viability and nutritional value [6, 7], and also causes quantitative and qualitative deterioration of pasta [8, 9]. Both the larvae and adult maize weevil cause significant damage to stored grain with 50% of the eggs laid during the first 5 weeks of the adult's life [10]. The female drills a hole in the grain and lays eggs inside before releasing a secretion that seals the cavity, protecting the eggs to complete their life cycle. Therefore, most insect control strategies focus on adult control [11]. Traditional management of *S. zeamais* populations is generally controlled with synthetic insecticides such as methyl bromide, phosphine, deltamethrin and chlorpyrifos-methyl [12]. Nerio et al. [13] reported that synthetic insecticides adversely affect human health and cause environmental pollution. Currently, research efforts are focused on finding alternative non-chemical, environmentally friendly strategies that can be used to reduce or eliminate *S. zeamais* in storage systems. Therefore, sourcing natural fumigants from plant extracts to control *S. zeamais* is actively pursued.

Recent research has turned toward selective biorational pesticides that are safer, cheaper and easier to produce than synthetic alternatives. Application of selected plant products as essential oils represents a simple, effective and relatively safe control method to manage populations of stored product insect pests [14, 15]. Several plant species applied as essential oils showed protective effects and toxicity against maize weevil, as well as antifeeding, repellence and progeny production [16-20]. Essential oil is a secondary metabolite produced by plants, and each plant produces an essential oil that contains different complexes. Plant essential oils contain some secondary compounds that can be used to kill pests, while plant extracts contain substances that have repellent properties that inhibit oviposition and show antifeedant, sterilization and toxicity to insects. Monoterpenoids kill insects by inhibiting the activity of acetylcholinesterase enzyme [21].

Carrot (*Daucus carota* L.) is a biennial plant of the Apiaceae family and one of the most commonly used root vegetables crops as food for human consumption. The taproot, as the edible part, is a nutritious and healthy food supplement with high vitamins, carotenoids, anthocyanins, fiber contents and other nutrients [22, 23]. Essential oil extracted from carrot seeds should not be confused with the cheaper softened carrot oil made by soaking carrot material in a base oil. The main chemical constituents of carrot oil extract include camphene, carotene, geranyl acetate, limonene, myrcene, sabinene, α-pinene, β-bisabolene, β-pinene and γ-terpinene [24]. In 1925, Asahina and Tsukamoto first isolated carotene pigment, one of the major components comprising 66% of essential oil from carrot seed. This sesquiterpene alcohol is formed in carrot seeds during the vegetation period and may be involved in allelopathic interactions expressing activity as an insecticidal, antifungal and herbicidal agent [24]. To the best of our knowledge, the insecticidal potentials of the essential oil of Thailand-grown *D. carota* seeds against maize weevil have never been reported. Thus, here, the insecticidal, fumigant toxicity and repellent activities of essential oil from seeds of *D. carota* were evaluated against adult maize weevil (*S. zeamais*).

2. Materials and methods

2.1 Insect rearing

Adult *S. zeamais* were collected from infested maize grains and reared following Wanna and Krasaetep [19] in a plastic container (30 cm diameter x 50 cm height). A total of 150 *S. zeamais* pairs were placed in a plastic container with 1 kg of maize kernels as food. The container was covered with a lid attached to a net and kept at room temperature of 30±5°C and 70±5% relative humidity with a 16L: 8D photoperiod in a laboratory at the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Maha Sarakham, Thailand. The weevils were allowed to mate and oviposit to increase their numbers and experimental consistency. Adult *S. zeamais* used for all tests were 7 days old.

2.2 Procurement and handling of essential oil of carrot seeds

Pure essential oil from carrot seeds, *D. carota* L., was purchased in an amber bottle from Botanicessence Essential Oils (Location M-Square Plaza, B1 Floor, Maleenon Building (Channel 3), Rama 4 Road, Bangkok, Thailand). The oil was kept in the dark at 4°C until required for bioassays.

2.3 Fumigation bioassay

Fumigation toxicity of essential oil from seeds of *D. carota* was tested on adult *S. zeamais* following the modified method of Wanna and Krasaetep [19]. The experiment was laid out in a completely randomized design (CRD) with four replications for four concentrations of essential oil (0 (control), 16, 32 and 64 µL/L air). Essential oil solutions were prepared by dilution with 100% acetone. Whatman (no.1) filter paper strips (1.5 cm width x 5 cm length) were saturated with 200 µL of essential oil solution, and the solvent was allowed to evaporate at room temperature for 2 min. The filter paper strips were hung in glass vials (2.5 cm diameter x 5 cm height) from the center of the fumigation bottle screw cap (5.5 cm diameter x 10.5 cm height) to avoid contact with the insects. Ten adult *S. zeamais* were transferred to each prepared fumigation bottle. The caps were tightened and the fumigation bottles were kept at 30±5°C and 70±5% relative humidity at a 16L:8D photoperiod. The control sets were fumigated with acetone alone. Adult mortality of *S. zeamais* was recorded at 24 to 120 h (Figure 1). An insect was considered dead when no movements of its legs or antennae were observed.

2.4 Repellent bioassay

The repellent effect of essential oil from seeds of *D. carota* on adult *S. zeamais* was evaluated using the vapor-phase with choice test following Satongrod and Wanna with slight modifications [25]. The experiment was laid out in CRD with four replications for three concentrations (8, 16 and 32 µL/L air) at 30±5°C and 70±5% relative humidity at a 16L:8D photoperiod. The repellent test kit consisted of two plastic bottles (each 8 cm diameter x 17 cm height) as a test bottle and an alternative bottle. A hole in the bottom of each bottle allowed the placement of a small plastic pipe (0.5 cm diameter x 15 cm length) as a connection between the test bottle and the alternative bottle. A hole was drilled in the middle of the plastic pipe with a sliding section that opened and closed to prevent insects from escaping. Essential oil solutions were prepared at three concentrations of 8, 16 and 32 µL/L air by dilution with 100% acetone. An aliquot of 200 µL of essential oil solution was dropped on a filter paper strip (1.5

cm width x 5 cm length) and evaporated at room temperature for 2 min. The filter paper was placed in a small glass vial (2.5 cm diameter, 5 cm height) and hung at the center of the test bottle cap with the lid closed tightly. For the alternative bottle, a filter paper was dropped with 200 µL of 100% acetone and prepared in the same way as the test bottle. Ten adult *S. zeamais* (7 days old) were released into a hole in the middle of the connecting plastic pipe between the test bottle and the alternative bottle and the hole was closed with a sliding pipe. The number of adult *S. zeamais* was recorded on the alternative bottles after the experiment at 24 to 120 h.

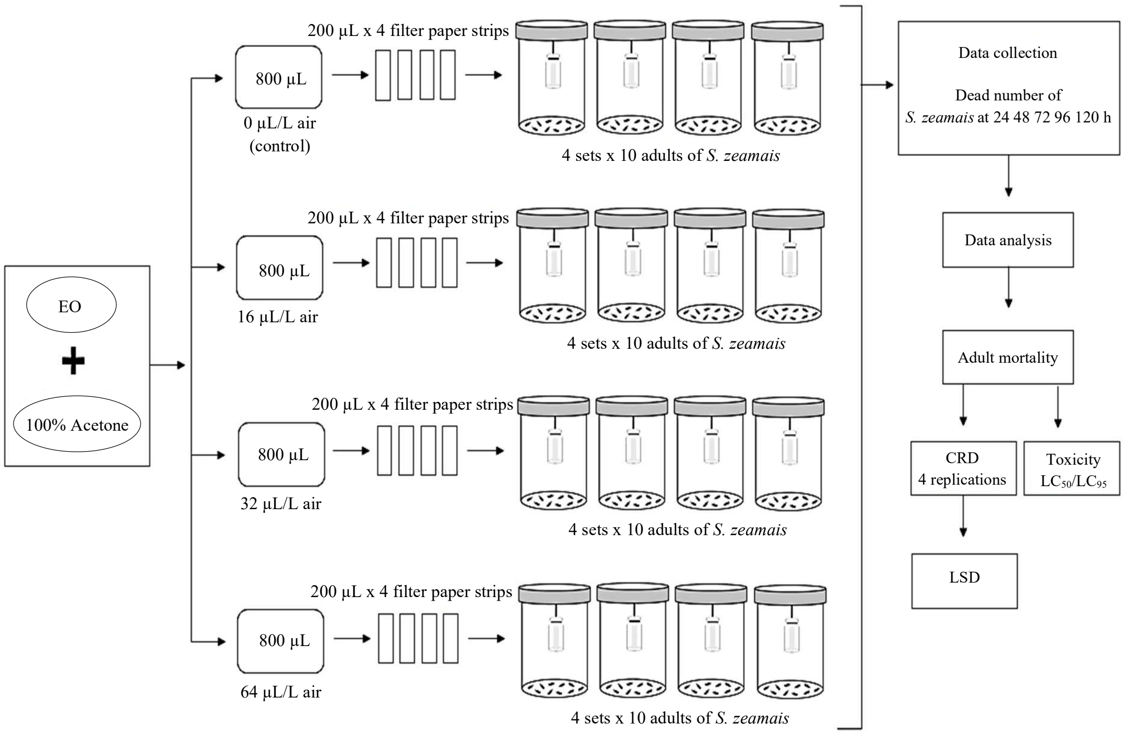

Figure 1 Experimental setup of the fumigation bioassay.

2.5 Data analysis

Mortality of *S. zeamais* was evaluated by the following equation %adult mortality = (Nd/Nt) x 100 where Nd is the total number of dead adult *S. zeamais* and Nt is the total number of adult *S. zeamais* used in the bioassay. Mortality data were adjusted for control mortality according to Abbott's formula [26]. The mortality in the control was between 5 and 20%. Fumigation toxicity of *D. carota* essential oil on adult *S. zeamais* was assessed for dose-mortality response using Probit analysis. The concentration of *D. carota* essential oil which is lethal to 50 percent (LC_{50}) and 95 percent (LC_{95}) of *S. zeamais* exposed in the time-period prescribed by the fumigant test were determined. The repellent effect was assessed using the repellence index (RI) as the formula $RI = 2T/(T+C)$ where T is the percentage of insects in the treatment bottle and C is the percentage of insects in the alternative bottle, with ranking values following Liu et al. [27]: $RI > 1$ gave an attractive effect, while $RI < 1$ produced a repellent effect. Mortality data and percentage repellent (PR) on adult *S. zeamais* were analyzed using the F-test by one-way analysis of variance (ANOVA). Mean values were compared using the least significant differences test (LSD) at a probability of ≤ 0.05.

3. Results and discussion

3.1 Fumigation toxicity bioassay

The LC_{50} and LC_{95} values of essential oil from *D. carota* seeds on adult *S. zeamais* assessed by the vapor phase test at 24 h were 26.27 and 67.75 µL/L air, respectively (Table 1). After 48 h exposure, LC_{50} and LC_{95} values were 19.60 and 54.72 µL/L air and 13.27 and 47.84 µL/L air at 72 h. *Daucus carota* seed essential oil showed significantly different fumigation activity on adult mortality of *S. zeamais* ($p < 0.01$). After 24, 48 and 72 h exposure, adult *S. zeamais* were susceptible to *D. carota* essential oil. The highest concentration of 64 µL/L air gave 100% adult mortality with significant difference ($p < 0.01$). (Table 2). When the duration was extended to 96 and 120 h, all concentrations of *D. carota* essential oil caused adult mortality of *S. zeamais* up to 100%.

Table 1 Fumigant toxicity of essential oil from seeds of *D. carota* on adult *Sitophilus zeamais*.

Time (h)	n	Linear regression Y = ax + b	LC_{50} (µL/L air)	LC_{95} (µL/L air)	r^2 value
24	160	y = 1.0848x + 21.5	26.27	67.75	0.69
48	160	y = 1.2812x + 24.89	19.60	54.72	0.70
72	160	y = 1.3016x + 32.724	13.27	47.84	0.58

n is the number of adult *S. zeamais* tested

LC_{50} means the concentration of essential oil from seeds of *D. carota* which is lethal to 50 percent of *S. zeamais* exposed in the time-period prescribed by the test

LC_{95} means the concentration of essential oil from seeds of *D. carota* which is lethal to 95 percent of *S. zeamais* exposed in the time-period prescribed by the test

Table 2 Adult mortality of *Sitophilus zeamais* exposed to essential oil from seeds of *D. carota*.

Concentration (µL/L air)	Mean (±SE) of adult mortality (%) of maize weevil				
	24 h	48 h	72 h	96 h	120 h
0	0.00±0.00 c	0.00±0.00 c	0.00±0.00 c	0.00±0.00	0.00±0.00
16	60.00±1.42 b	71.40±1.16 b	86.90±0.61 b	100.00±0.00	100.00±0.00
32	67.50±1.26 ab	76.70±0.67 b	89.70±0.60 b	100.00±0.00	100.00±0.00
64	80.00±0.82 a	95.00±0.58 a	100.00±0.00 a	100.00±0.00	100.00±0.00
F-test	**	**	**	N/A	N/A
LSD	15.881	11.199	4.726	-	-
CV%	19.87	11.96	4.44		

SE (Standard Error).

N/A (Not Applicable).

** represents significant difference at $p < 0.01$.

Means within the same column followed by the same letter show significant difference at $p > 0.05$ by LSD.

Generally, the fumigation method is used to control insect pests in storage products because this method is inexpensive, convenient, fast and effective against insect pests of all life stages [28]. A major characteristic of essential oil as a pesticide formulation is that it is effective even without direct contact with the target organism [29]. However, the fumigant agents currently used as methyl bromide and phosphine are highly toxic to humans [30, 31]. Thus, using a natural fumigant agent from *D. carota* seeds for control of adult *S. zeamais* is a promising alternative. Staniszewska and Kula [24] reported that the main chemical compositions of *D. carota* essential oil included camphene, carotene, geranyl acetate, limonene, myrcene, sabinene, α-pinene, β-bisabolene, β-pinene and γ-terpinene, while Yildirim et al. [32] assessed the insecticidal effects of several monoterpenes by fumigation toxicity against *S. zeamais*. The monoterpene hydrocarbon limonene reduced survival by up to 79% in *S. zeamais* after 96 h, while camphene, β-pinene and α-pinene induced 50% insect mortality during the same period. Monoterpene hydrocarbons were highly effective against adult *S. zeamais* and contributed significantly to the fumigant toxicity of the essential oil. Our results concurred with Yildirim et al. [28] who reported insecticidal activities of some monoterpenes, while Ogendo et al. [33] suggested that cell changes and blocking of octopamine receptors, a neuromodulator/neurohormone present in all invertebrates, may be the mechanism of fumigation toxicity from terpenoid compounds in the essential oils.

3.2 Repellent bioassay

No significant differences for repellent activity were recorded in adult *S. zeamais* at all concentrations ($p > 0.05$) (Table 3). Essential oil from seeds of *D. carota* showed percentage repellent (PR) on adult *S. zeamais* between 12.50 and 45.00%, while 32 µL/L air of *D. carota* essential oil at 120 h provided the highest PR on adult *S. zeamais* at 45.00±3.11%. All concentrations of essential oil from seeds of *D. carota* at 24 to 120 h showed no repellent effect on adult *S. zeamais*. By contrast, they showed an attractive effect on adult *S. zeamais*, with repellence index value of more than 1 (Table 4). The concentration of 16 µL/L air of *D. carota* essential oil showed a high repellence index of between 1.5 and 1.8 at all testing durations.

Table 3 Repellent activity of adult *Sitophilus zeamais* exposed to essential oil from seeds of *D. carota*

Concentration (µL/L air)	Mean (±SE) of repellent (%) of maize weevil				
	24 h	48 h	72 h	96 h	120 h
8	25.00±1.29	22.5±0.96	35.00±1.29	40.00±2.83	40.00±2.75
16	25.00±1.29	27.5±1.26	17.50±1.50	12.50±1.26	15.00±1.29
32	32.50±1.26	37.5±1.71	40.00±3.37	42.50±2.75	45.00±3.11
F-test	ns	ns	ns	ns	ns

SE (Standard Error).

ns (non-significant).

Essential oil from seeds of *D. carota* did not act as a repellent to adult *S. zeamais*. Adult *S. zeamais* detected the presence of essential oil but did not interpret this as a cue to avoid exploitation of the site. The lack of repellent effects of the essential oil from seeds of *D. carota* may be linked to its relatively high volatility, similar to the essential oil of *Evodia rutaecarpa* Benth. which lost repellent activity after evaporation at 5 min or longer [34].

Table 4 Repellent index of essential oil from seeds of *D. carota* against adult *Sitophilus zeamais*.

Time (h)	Concentration (µL/L air)	RI (Repellence index)	Meaning
24	8	1.5±0.3	attractive effect
	16	1.5±0.3	attractive effect
	32	1.4±0.3	attractive effect
48	8	1.6±0.2	attractive effect
	16	1.5±0.3	attractive effect
	32	1.3±0.3	attractive effect
72	8	1.3±0.3	attractive effect
	16	1.7±0.3	attractive effect
	32	1.2±0.7	attractive effect
96	8	1.2±0.6	attractive effect
	16	1.8±0.3	attractive effect
	32	1.2±0.6	attractive effect
120	8	1.2±0.5	attractive effect
	16	1.7±0.3	attractive effect
	32	1.1±0.6	attractive effect

RI (Repellence Index) > 1 indicates attractive effect and RI < 1 indicates repellent effect

4. Conclusion

Essential oil from seeds of *D. carota* showed potential for control of *S. zeamais*, with toxicity to adult insects by fumigation. The insecticidal effect could be due to monoterpene activity, previously reported as insecticides against *S. zeamais*. No repellent effect was shown, and this essential oil had an attractive effect on adult maize weevils. Therefore, it could be developed and applied as a decoy in traps for capturing adult maize weevils. Synthetic essential oils can be used to eliminate insect pests in storage products. However, comprehensive studies of other effects like population growth deterrence are required. *D. carota* seed essential oil requires further evaluation of future formulations for *S. zeamais* control.

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