Insecticidal Activity of Essential Oil from Seeds of *Foeniculum vulgare* (Apiales: Apiaceae) Against *Sitophilus zeamais* (Coleoptera: Curculionidae) and Its Effects on Crop Seed Germination[1]

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Abstract Many aromatic herbs in the family Apiaceae produce essential oils that are used on an industrial scale for medicinal, cosmetic, and food purposes. Essential oils from plants that show insecticidal activity can be substituted for synthetic insecticides to reduce environmental pollution or harmful toxicity to humans. Insecticidal activity of essential oil from seeds of coriander, *Foeniculum vulgare* Miller (Apiales: Apiaceae), on maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), and its effects on seed germination of three major economic crops were investigated. Essential oil from *F. vulgare* seeds was analyzed by gas chromatography–mass spectrometry and found to contain 25 components, with anethole (43.78%) as the main constituent. Other basic compounds were estragole (28.33%), fenchone (16.68%), d-limonene (2.62%), alpha-pinene (1.84%), and p-cymene (1.28%). *Foeniculum vulgare* essential oil was toxic to maize weevil when used as a fumigant with a median lethal concentration at 48 h of 10.42 μL/L air. A concentration of 64 μL/L air was most effective, killing 100% of maize weevil within 24 h. The repellent effect on maize weevil increased to 75% at 24 h at a concentration of 16 μL/L air. Rice (*Oryza sativa* L.) and sorghum (*Sorghum bicolor* [L.] Moench) seeds fumigated with 32 μL/L air of *F. vulgare* essential oil exhibited a slight decrease in germination rate (germination $>$90%). Our results indicate that essential oil from *F. vulgare* seeds have potential for application in the management of maize weevil in stored products.

Key Words chemical composition, toxicity, insecticide, stored-product insect pest, germination rate

Infestation by weevils (Coleoptera: Curculionidae) compromises the quality and quantity of stored products. The use of methyl bromide has been reduced and phased out in some countries due to environmental impacts. Several physical, chemical, and biological control methods are used to prevent and eliminate damaging insect pests in stored products. Globally, the most commonly used chemicals to control insect pests are synthetic pesticides and fumigants (Wasala et al. 2016) including phosphine fumigants and seed-infusing chemicals. There is growing concern about the negative effects of synthetic insecticides, including their

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direct toxicity to the user, residual toxicity in the environment, increasing levels of insect pest resistance and resurgence, and toxicity to nontarget organisms (Isman 2006). Therefore, alternative management methods must be identified and developed as substitutes for synthetic insecticides (Kaan et al. 2016, Kostyukovsky et al. 2016).

One such avenue of research is with plant-derived extracts. Essential oils are a complex combination of a large number of varying proportions of chemical constituents that act as contact toxins, fumigants, repellents, antifeedants, ovicides, or attractants, or have other effects on insects (Regnault-Roger and Hamraoui 1995, Papachristos and Stamopoulos 2002, Isman 2006, Werdin-González et al. 2008). Isman (2000) and Gutiérrez et al. (2015) reported neurotoxic, cytotoxic, phototoxic, and mutagenic activities of essential oils on insects. They may interfere with metabolism, biochemistry, physiology, and behavior (Mann and Kaufman 2012) and can also inundate the respiratory tracts and lead to asphyxiation and death (Liang et al. 2013).

Plant essential oils are potential alternatives to conventional synthetic insecticides (Koutsaviti et al. 2017, Liang et al. 2013, Utono and Gibson 2015). Some of the advantages of their use as botanical insecticides are their low cost, ease of use, lack of negative effects on human health, environmental compatibility, and ease of biodegradability (Isman 2006). Indeed, essential oils have been studied for management of insect pests in stored grains (Rajendran and Srianjini 2008, Wanna 2021, Wanna et al. 2021, Wanna and Satongrod 2020, Wanna and Wongsawas 2022, Wuttiwong et al. 2015). We undertook this study to determine the insecticidal efficacy of an essential oil extracted from the seeds of coriander, *Foeniculum vulgare* Miller (Apiales: Apiaceae), against the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). We also examined the effect of this essential oil on the germination of rice (*Oryza sativa* L. [Poales: Poaceae]), maize (*Zea mays* L. [Cyperales: Poaceae]), and sorghum (*Sorghum bicolor* [L.] Moench [Poales: Poaceae]).

Materials and Methods

Insects. Adult maize weevils were collected from infested maize in storage in Kantharawichai District, Maha Sarakham Province, Thailand. They were transported to the laboratory where they were maintained in plastic containers measuring 30 cm in diameter (diam.) and 50 cm high. The maize kernels used for rearing the weevils were frozen for 12 h to kill any associated pests or contaminants. One kilogram of maize kernels was placed in a plastic container, and 100 adult maize weevils were added. The plastic container was covered with a lid with a net for ventilation and kept at 30 ± 5°C and 70 ± 5% relative humidity on a 16:8 h (light:dark) photoperiod regime at the Department of Agricultural Technology, Mahasarakham University (Maha Sarakham, Thailand). Twenty pairs of maize weevils were separated into a plastic container with 250 g of maize kernels. These weevils were allowed to mate and oviposit for 5 d. Members of the parental generation were removed, and the newly emerged progeny (aged 7 d) were used for further bioassays. All experiments were conducted in a completely random design with four replications at the same environmental conditions described for insect rearing.

Essential oil. Pure essential oil from *F. vulgare* seeds was purchased in an amber bottle from Botanicessence Essential Oils (Bangkok, Thailand). The oil was kept at 4°C in the dark until used for analyses and bioassays. Composition of the essential oil was confirmed by gas chromatography–mass spectrometry analysis following the methods of Satongrod and Wanna (2020) using a Clarus 680 gas chromatograph (PerkinElmer, Inc., Shelton, CT) equipped with a Rtx-5MS capillary column (5% phenyl-methylpolysiloxane stationary phase, 30.0 × 0.32mm internal diam., 1.0μm film thickness) and mass detector turbo mass operated in EI mode. Helium was the carrier gas used at a flow rate of 1.0 mL/min. One microliter (100,000 ppm) of the individual samples was injected with split mode (split ratio of 1:100 v/v). The injector temperature was operated at 200°C, and the oven temperature was programmed as follows: 45°C for 5 min, then increased to 280°C at a rate 10°C/min and held for 5 min, functioning in electron impact mode of 70 eV. A mass analyzer was used as a quadrupole, and the temperature detector was set at 250°C. Spectra were scanned (m/z) from 40 to 1,000 atomic mass units (amu). The essential oil components were identified by comparing their mass spectra with those in the database of the National Institute of Standards and Technology Mass Spectral Search Program (Gathersburg, MD) and the ChemStation Wiley Spectral Library (Scientific Instrument Services, Palmer, MA) with a quality match >80%. Chemical composition data of the essential oil from *F. vulgare* seeds were identified by retention time and percentage of area within the chromatogram.

Fumigation toxicity. Fumigation toxicity of the essential oil from *F. vulgare* seeds was tested against adults of the maize weevil following a modified method of Wanna and Krasaetep (2019). Briefly, Whatman (no. 1) filter paper strips (1.5 cm wide, 5 cm long) were saturated with 200 μL of 0 (control), 16, 32, and 64 μL/L air of *F. vulgare* essential oil, diluted with 100% acetone. The solvent was allowed to evaporate for 2 min at room temperature. Filter paper strips were suspended in glass vials (2.5 cm diam., 5 cm high) at the center of the screw cap of the fumigation bottle (5.5 cm diam., 10.5 cm high) to avoid contact with the insects. Ten adult maize weevils (7 d old) were transferred to each fumigation bottle. The caps were screwed tightly shut and the bottles were kept at previously described conditions. Controls were fumigated with 100% acetone alone. The adult mortality of maize weevils was recorded at time intervals between 24 to 168 h after treatments. Maize weevil adults were considered dead when no leg or antenna movements were observed.

Repellent effect. The repellent effect of *F. vulgare* essential oil against maize weevil was assessed by the vapor phase with choice test following slightly modified methods of Satongrod and Wanna (2020). A repellent test kit consisted of two plastic bottles (each bottle, 8 cm diam., 17 cm high) as a test bottle and an alternative bottle. A hole at the lower side of each bottle allowed placement of a small plastic tube (0.5 cm diam., 15 cm long) as a connection between the test bottle and the alternative bottle. A hole was drilled in the middle of the tube for adult maize weevil release, with a sliding tube in the center that opened and closed to prevent escape. Essential oil solutions were prepared at three concentrations of 8, 16, and 32 μL/L air by dilution with 100% acetone. An aliquot of 100 μL of the essential oil solution was released on the filter paper strip (1.5 cm wide, 5 cm long)

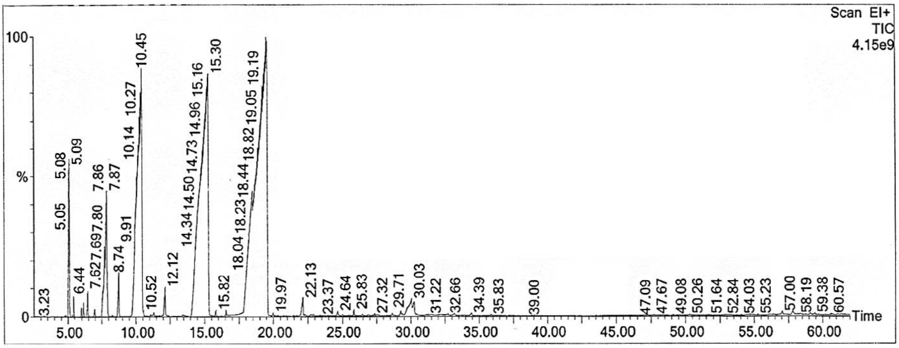

Fig. 1. Chromatogram of the chemical components of essential oil from *F. vulgare* seeds.

and evaporated at room temperature for 2 min. A filter paper strip was placed in a small glass vial (2.5 cm diam., 5 cm high) and suspended from the center of the screw cap of the test bottle (8 cm diam, 17 cm high) with the screw cap tightly closed. For the alternative bottle, a filter paper strip was saturated with 100 μL of 100% acetone and prepared in the same way as the test bottle. Ten adult maize weevils were released into the opening in the middle of the connecting tube between the test bottle and the alternative bottle, and the sliding tube was tightly closed. Numbers of maize weevil adults were recorded in the test bottle and the alternative bottle at time intervals between 24 to 168 h.

Seed germination effect. Germination of the seeds of three major crops—rice (*O. sativa*), maize (*Z. mays*), and sorghum (*S. bicolor*)—was determined following exposure of the seed to the essential oil at the concentrations tested in the fumigation and repellence tests. For each plant species and mode of exposure (fumigation vs repellence), 100 seeds were arranged in rows at regular intervals on two layers of moistened filter paper and then covered with another layer of moistened filter paper. The filter papers with the seeds were folded and loosely rolled. Each of these rolls of filter paper with seed represented a replicate. For each treatment (plant species, fumigation versus repellence exposure, essential oil concentration), four rolls (400 seeds total) were established and placed in a plastic bag which was then closed and sealed with rubber bands to prevent moisture loss. All bags were placed vertically in a deep plastic tray. These were maintained at the same environmental conditions described for insect rearing. After 5 d, the filter paper rolls were opened, and the germinated seeds counted. Germination was expressed as a percentage of the total ($n = 100$) seeds in each replicate.

Statistical analyses. Mortality of maize weevil was evaluated by the equation, % adult mortality = (Nd/Nt) × 100, where Nd is the number of dead maize weevil adults and Nt is the total number of maize weevil adults used in the bioassay. Mortality data were adjusted for control mortality according to Abbott's (1925) formula when mortality in the control ranged between 5 and 20%. Fumigation toxicity of *F. vulgare* essential oil on maize weevil adults was assessed for concentration-mortality response using probit analysis (Finney 1971) yielding median lethal concentration (LC_{50}) and 90% lethal concentration (LC_{90}) values and

Table 1. Chemical composition of essential oil from *F. vulgare* seeds.

No.	Compound	Retention Time (min)	% Area
1	alpha-Pinene	5.091	1.84
2	Camphene	5.429	0.15
3	alpha-Phellandrene	5.997	0.16
4	alpha-Myrcene	6.455	0.34
5	p-Cymene	7.714	1.28
6	d-Limonene	7.869	2.62
7	ç-Terpinene	8.742	0.55
8	Fenchone	10.451	16.68
9	Linalool	11.301	0.05
10	Camphor	12.117	0.40
11	Estragole	15.305	28.33
12	Fenchol	15.823	0.05
13	4-Methoxybenzhydrazide	17.637	0.13
14	Anethole	19.511	43.78
15	4-Methoxybenzhyl isothiocyanate	22.135	0.31
16	(S,Z)-2-Methyl-6-(p-tolyl)hept-2-en-1-ol	25.836	0.07
17	Benzoic acid, 4-methoxy-,4-ethylphenyl ester	28.651	0.04
18	Trans-4-methoxycinnamaldehyde	29.256	0.07
19	4-Methoxybenzyl alcohol, isopropyl ether	30.039	0.75
20	Succinic acid, di(4-methoxybenzyl) ester	30.232	0.36
21	4-Methoxybenzyl alcohol, n-propyl ether	33.170	0.06
22	1-Hydroxy-1-(4-methoxyphenyl)propan-2-one	34.443	0.05
23	2,4-Bis(4-methoxyphenyl)-3,5-dimethyltetrahydrofuran	57.037	0.08
24	Diethylstilbestrol	57.795	0.09
25	5-(2-Aminopropyl)-2-methylphenol	59.076	0.03
	Total		98.26

associated parameters. 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. (2007): $RI > 1$ was an attractive effect, while $RI < 1$ produced a repellent effect. Seed germination was calculated with the following

Table 2. Concentration-mortality response of adult maize weevils to the essential oil extracted from *F. vulgare* seeds.

Time (h)	*n*	LC_{50} (95% CL) (μL/L air)[a]	LC_{90} (95% CL) (μL/L air)	df	χ^2
24	280	16.43 (12.52-21.21)	44.25 (36.12-58.49)	26	278.43
48	280	10.42 (4.89-15.76)	41.12 (31.64-61.36)	26	391.43

[a] CL represents confidence limit; LC_{50}, median lethal concentration; LC_{90}, 90% lethal concentration.

formula: germination = (number of germinated seeds/total number of seeds) × 100. Toxicity, repellence, and seed germination data were analyzed using the one-way analysis of variance. Treatment means were compared using the least significant differences test at $P \leq 0.05$ using Statistix, version 9.0 (Analytical Software, Tallahassee, FL).

Results

Chemical composition of *F. vulgare* essential oil. Our chemical analysis identified 25 compounds (98.26%) in the *F. vulgare* seed essential oil. Six components (94.53%) had percentage of area >1% based on the highest peak, classified using the International Union of Pure and Applied Chemistry system. The highest percentage of area was anethole (43.78%), followed by estragole (28.33%), fenchone (16.68%)), D-limonene (2.62%), alpha-pinene (1.84%), and p-cymene (1.28%) (Fig. 1, Table 1).

Fumigation toxicity. At 24 h, the *F. vulgare* essential oil had an LC_{50} of 16.43 μL/L air, while the LC_{50} at 48 h was 10.42 μL/L air (Table 2). The essential oil at 16 μL/L yielded a mean ± SD adult mortality of 45.0 ± 12.9% to 90.0 ± 0.0%, while mortality at 32 μL/L air was 70.0 ± 8.2% to 95.0 ± 5.8% (Table 3). At 168 h, there were no significant differences in adult mortality among all treatments. The essential oil at 64 μL/L air had the highest mortality at 100% in all test periods.

Repellent effect. Based on the RI values (mean ± SD), *F. vulgare* essential oil at all three concentrations repelled maize weevil adults at 24 h (Table 4). However, the essential oil was largely attractive to the maize weevils from 48 through 168 h. The highest concentration tested (32 μL/L air) repelled the weevils at 120, 144, and 168 h. Yet, in assessing the percentage of adult weevils repelled by the essential oil, there were no statistical differences among the treatment means within each time interval (Table 5).

Seed germination. Germination did not differ among the concentrations tested when the seeds were exposed by fumigation (Table 6), with mean germination of 97.2–98.5% for rice, 73.0–78.5% for maize, and 92.0–95.5% for sorghum, with no significant differences when compared with the 0 μL/L air control. The essential oil applied as a repellent had no significant impact on maize seed germination, while the highest concentration (32 μL/L air) significantly reduced the germination of rice and sorghum in comparison to the control (Table 6).

Table 3. Mean ± SD mortality of adult maize weevil after fumigation with essential oil extracted from *F. vulgare* seeds.[a]

Concentration (μL/L air)	Adult Mortality (%) of Maize Weevil						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
0	0.0 ± 0.0d	0.0 ± 0.0d	0.0 ± 0.0d	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0d	0.0 ± 0.0
16	45.0 ± 12.9c	60.0 ± 8.2c	72.5 ± 9.6c	82.5 ± 9.6b	85.0 ± 5.8b	90.0 ± 0.0c	100.0 ± 0.0
32	70.0 ± 8.2b	72.5 ± 9.6b	80.0 ± 0.0b	87.5 ± 5.0b	90.0 ± 0.0b	95.0 ± 5.8b	100.0 ± 0.0
64	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0
F-test	**	**	**	**	**	**	N/A†
LSD	11.76	9.69	7.37	8.32	3.85	4.44	—

[a] Means within the same column followed by the same letter are not significantly different (LSD: $P > 0.05$).

** Significant difference at $P \leq 0.01$.

† NA represents not applicable; LSD, least significant difference.

Table 4. Repellency index (RI ± SD) of adult maize weevil by exposed to the essential oil extracted from *F. vulgare* seeds.

Time (h)	Concentration (μL/L air)	RI[a]	Reaction
24	8	0.9 ± 0.3	Repellence
	16	0.5 ± 0.3	Repellence
	32	0.6 ± 0.2	Repellence
48	8	1.3 ± 0.2	Attraction
	16	1.3 ± 0.2	Attraction
	32	1.2 ± 0.3	Attraction
72	8	1.2 ± 0.4	Attraction
	16	1.3 ± 0.2	Attraction
	32	1.3 ± 0.4	Attraction
96	8	1.1 ± 0.2	Attraction
	16	1.3 ± 0.2	Attraction
	32	1.4 ± 0.3	Attraction
120	8	1.1 ± 0.1	Attraction
	16	1.0 ± 0.2	Attraction
	32	0.8 ± 0.2	Repellence
144	8	1.1 ± 0.1	Attraction
	16	1.2 ± 0.2	Attraction
	32	0.8 ± 0.2	Repellence
168	8	1.2 ± 0.2	Attraction
	16	1.2 ± 0.2	Attraction
	32	0.8 ± 0.2	Repellence

[a] RI >1 showed attraction and RI <1 showed repellence.

Discussion

Shamkant et al. (2014) reported that *F. vulgare* has chemical components such as phenylpropanoids, monoterpenes, and sesquiterpenes with trans-anethole being the most abundant. These compounds have exhibited broad-spectrum insecticidal activity against a variety of insects due to the presence of diverse active molecules, each with a different mode of action (Camarillo et al. 2009, Gillette et al. 2009, Shamkant et al. 2014). Anethole was previously identified for its role in insect pest control (Xu et al. 2012, Zoubiri et al. 2014). Some compounds identified in the essential oil from *F. vulgare* seeds showed insecticidal synergistic activity between

Table 5. Mean ± SD percentage of adult maize weevils repelled following exposure to the essential oil extracted from *F. vulgare* seeds.

Concentration (µL/L air)	Adult Repellence (%) of Maize Weevil						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
8	55.0 ± 9.6	37.5 ± 9.6	40.0 ± 21.6	47.5 ± 9.6	45.0 ± 5.8	45.0 ± 5.8	40.0 ± 8.2
16	75.0 ± 9.6	37.5 ± 9.6	37.5 ± 9.6	37.5 ± 9.6	52.5 ± 9.6	42.5 ± 9.6	42.5 ± 9.6
32	70.0 ± 17.1	42.5 ± 17.1	35.0 ± 19.2	32.5 ± 17.1	60.0 ± 8.2	60.0 ± 8.2	62.5 ± 9.6
F-test	ns[a]	ns	ns	ns	ns	ns	ns

[a] ns represents nonsignificant difference at $P > 0.05$.

Table 6. Mean ± SD percentage of germination of *O. sativa*, *Z. mays*, and *S. bicolor* seeds exposed by application of the essential oil extracted from *F. vulgare* seeds either as a fumigant or as a repellent.[a]

Application Method	Concentration (μL/L air)	*O. sativa*	*Z. mays*	*S. bicolor*
Fumigation	0	98.5 ± 1.3	78.5 ± 5.0	95.5 ± 0.6
	16	97.2 ± 2.1	74.5 ± 8.5	95.0 ± 1.4
	32	97.2 ± 1.3	74.0 ± 3.3	93.5 ± 3.1
	64	97.2 ± 1.7	73.0 ± 6.0	92.0 ± 2.9
	F-test	ns[b]	ns	ns
Repellent	0	98.5 ± 1.3a	78.5 ± 5.0	95.5 ± 0.6a
	8	98.0 ± 0.8a	77.0 ± 5.3	92.5 ± 5.0ab
	16	97.2 ± 1.0ab	74.5 ± 4.4	90.8 ± 3.1ab
	32	96.0 ± 1.2b	74.0 ± 5.9	90.2 ± 2.8b
	F-test	*	ns	*

[a] Means within the same column followed by the same letter are not significantly different (LSD: $P > 0.05$).
[b] ns represents nonsignificant difference at $P > 0.05$.
* Significant difference at $P \leq 0.05$.

major and minor constituents. Plants with essential oils exhibiting such activity have been proposed as alternative crops to integrate a strategy for more extensive use of natural substances in the field (Tak and Isman 2015).

Foeniculum vulgare essential oil exhibited fumigation toxicity to adult maize weevil, consistent with Ebadollahi (2011) who reported that extracts from *F. vulgare* (Apiaceae) were toxic to *Sitophilu*s *granarius* (L.) and *Sitophilu*s *oryzae* (L.) as fumigants, while Işik and Görür (2009) reported that *F. vulgare* essential oil had strong insecticidal activity with the potential to prevent aphid*, Brevicoryne brassicae* (L.), infestations. Our results demonstrated that *F. vulgare* essential oil also was effective in fumigating adult maize weevil. This result was consistent with Kim and Ahn (2001) who reported that *F. vulgare* essential oil from seeds was effective as a fumigation agent with mortality of 100%. Essential oil extracted from *F. vulgare* seeds showed a positive relationship between concentration and mortality. A consistent relationship was shown between mortality and bioassay concentration of several insects with various essential oils (Heydarzade and Moravvej 2012).

Our results demonstrating that the repellency of the *F. vulgare* essential oil against maize weevil adults seemingly contradicts the findings of Gusmão et al. (2013), who reported that *F. vulgare* essential oil had no effect as a fumigant (RI < 1) against cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae). Our determination of the repellency of *F. vulgare* essential oil against maize weevil adults was at 70%, similar to the findings of Araújo et al. (2019) who determined that *F. vulgare* essential oil was 77.1% effective. These essential oils were toxic and/or repellent to adult maize weevil, and their activity appeared concentration-dependent. The effectiveness of essential oil extracts also was influenced by their persistence in the environment over time (Cubillo et al. 1999).

Foeniculum vulgare essential oil from seeds show great potential as an alternative to synthetic insecticides currently used to control insect pests in grains. The essential oil used as a fumigant or as a repellent had little to no effect on germination of rice, maize, or sorghum seed. Given that essential oils have variable compositions, further studies are necessary to clearly identify the compound(s) responsible for insecticidal toxicity of this essential oil to avoid variable treatment responses. Action mechanisms of this essential oil on insects and mammals, including the role of minor components, require clarification before expansion to industrial use.

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