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Toxicity and bioactivity of essential oil of Cilantro (*Eryngium foetidum* L.) against red flour beetle [*Tribolium castaneum* (Herbst)]

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Abstract

Grains and milling products are a major part of the daily diet of humans and animals but grain storage is heavily impacted by insect pests. Toxicity and bioactivity of essential oil (EO) from Cilantro, *Eryngium foetidum* L. were evaluated against the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), known as one of the most common stored-product insect pests worldwide. EO was extracted from fresh leaves of *E. foetidum* using a Clevenger type apparatus, and its toxicity and bioactivity against *T. castaneum* were investigated using the topical application test, the vapor phase test, and the impregnated paper test. Toxicity of *E. foetidum* EO on *T. castaneum* adults was investigated by direct contact and fumigation bioassays. *E. foetidum* EO was efficient with low estimated LD_{50} and LC_{50} as 31.34 µL/mg adult and 13.03 µL/L air, respectively. Insecticidal efficiency of *E. foetidum* EO on *T. castaneum* adults showed that a dosage of 25 µL/mg adult and a concentration of 32 µL/L air killed 62.5% after 120 h of exposure and 72.50% after 72 h of exposure. *E. foetidum* EO was strongly repellent to *T. castaneum* adults at 2.5 µL/L air, with highest repellent activity (100%) after 7 h of exposure. Results indicated that *E. foetidum* EO showed potential as a bio-insecticide for the control of *T. castaneum*.

Keywords: toxicity; insecticide; repellent; stored-product insect pests; essential oil.
Abbreviations: Conc._concentration; EO_essential oil; LD_lethal dose; LC_lethal concentration.

Introduction

Grains are often contaminated and destroyed by insect pests during storage, posing a serious threat to both farmers and consumers (Jian, 2019). Insects that infest various stored grain products are noted for their strong reproductive ability. Damage resulting from insect pests includes reduction of stored grain products, nutritional value, and contamination (Ebadollahi and Sendi, 2015). Insect pests cause serious food grain losses in storage, especially at farm level in tropical and subtropical countries. About 100 insect species of the family Tenebrionidae (Order: Coleoptera) are known to cause post-harvest damage of stored food commodities. The red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) is a secondary insect pest; it cannot penetrate and cause damage to grains and needs a substrate that has been previously damaged by primary pests. *T. castaneum* is a highly successful insect pest for stored food products in the wet and dry climate of tropical areas (Jayas et al., 1995), and causes high losses to stored food including grain, seed, flour and milling products (Arthur et al., 2019). Adult *T. castaneum* are reddish-brown, with a lifecycle of five to six months under optimal conditions of 35°C and 75% relative humidity. They can survive temperature ranges of 22-40°C and have the fastest reproductive rate among stored-product insect pests, increasing their number by up to 70-100 times in a month (Devi and Devi, 2015). Both larvae and adults cause severe infestation and contaminate products with their dead bodies and fecal materials; the flour turns grayish with mold growth. *T. castaneum* releases the defensive secretion benzoquinone, which is widely known as a cancerogen (Unruh et al., 1998), and its disagreeable odor renders it unfit for human consumption. *T. castaneum* may also induce an allergic response, while intake of contaminated food causes serious health hazards to humans and livestock. Stored food commodities infested by *T. castaneum* undergo qualitative decrements along with quantitative losses.

Several control methods related to physical, chemical and biological combat have been used against harmful insects in stored products. Globally, the most commonly used chemicals to control anti-crop agents that act in storage facilities are synthetic insecticides and fumigants (Wasala et al., 2016). However, increasing concern has been expressed about the negative effects of synthetic chemical insecticides including their direct toxicity to users, excessive usage, residual toxicity in the environment, increasing level of insect resistance to insecticides and insect pest resurgence, and toxicity to non-target living organisms (Isman, 2006). Because of these negative effects, alternative methods have been considered. To protect the environment and avoid adverse ecological impacts, research efforts have focused on new methods of insect pest management in grain stores, with attention diverted toward the use of organic products as pesticides, such as plant extracts (Rajendran and Sriranjini, 2008; Wanna et al., 2018; Wanna and Satongrod, 2020).

The Apiaceae (Umbelliferae) family consists of aromatic plants that are used as food and spices and in pharmacy, agriculture and cosmetics (Ebadollahi, 2013). The Apiaceae are a valuable source of essential oils that can be extracted by steam or water distillation from the flowers, seeds, leaves, stems, bark and roots of plants. Major constituents of Apiaceae essential oils are monoterpenes, sesquiterpenes and phenylpropanoids (Cianfaglione et al., 2017). The genus *Eryngium* is an important member of the family Apiaceae and consists of some 317 species distributed in temperate regions of every continent (Wörz, 2004; Medbouhi et al., 2018). This genus contains pharmaceutical plants based on their phytochemical composition and pharmacological potential (Wang et al., 2012). Phytochemical studies have been performed on *Eryngium* species containing several secondary substances such as terpenoids, steroids, triterpenoid saponins, polyacetylenes, phenolics, flavonoids, coumarins and rosmarinic acid derivatives (Marčetić et al., 2014; Bouzergoune et al., 2016). Antioxidant, cytotoxic, anti-inflammatory, antimicrobial, antimalarial, antidiabetic and anti-mutagenic activities from *Eryngium* species have also been reported. (Ural et al., 2014; Benmerache et al., 2016).

Eryngium foetidum L. is known by many common names in different languages such as shado beni (Trinidad), chadron benee (Dominica), fitweed (Guyana), coulante or culantro (Haiti), recao (Puerto Rico) langer coriander (German), ketumbar java (Malay), pak chi farang (Thai), ngo gai (Vietnamese), culantro, racao, recao (Spanish), bhandhanya (Hindi), jid yudn qidn (Chinese) and long leaf or spiny coriander (English) (Eyres et al., 2006; Singh et al., 2006; Chowdhury et al., 2007; Paul et al., 2011). This herb can be used instead of coriander because it has a similar pungent odor (Chowdhury, 2007). Cilantro has been recommended as a health food because the plant contains significant amounts of calcium, iron, carotene, riboflavin, protein, vitamins A, B and C and essential oils in the aerial parts (Aly, 2010). *E. foetidum* has been evaluated for its anti-convulsant and anthelmintic properties, while its extract is a potential source of natural products with topical anti-inflammatory activity (Sáenz et al., 1997; García et al., 1999). Cilantro also contains essential volatile substances and is an important perfume in the cosmetic industry (Paul et al., 2011).

Essential oils are secondary metabolites that are present in all parts of plants. They are complex compounds and contain many components that determine their properties including terpenes, aromatic and aliphatic compounds. The main terpenes are monoterpenes and sesquiterpenes (Bakkali et al., 2008; Koul et al., 2008). Monoterpenes comprise 90% of the essential oils, and are represented by compounds with different structures such as acyclic (geraniol) and cyclic (terpineol) spirits, phenols (thymol), ketones (thujone), aldehydes (citronellal), acids (chrysanthemic acid) and oxides (1,8-cineole). Aromatic compounds such as cinnamaldehyde, chavicol, anethole, safrole and apiole are derivatives of phenylpropane and are present in lower amounts (Isman, 2006; Tripathi et al., 2009). Essential oils obtained from plants are complex mixtures of a large number of chemical constituents in variable proportions that cause acute toxicity and have fumigant and repellent effects, preventing nutrition and limiting development with antifeedant, ovicidal, and other adverse 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 activity of essential oils on insects. Essential oils affect the main metabolic, biochemical, physiological and behavioral functions of insects (Mann and Kaufman, 2012), and can also block respiratory tracts leading to asphyxiation and death of pests (Kaufmann and Briegel, 2004; Rotimi et al., 2011). Advantages of essential oils include low cost, ease of use and availability, and lack of negative effects on human health; they are also environmentally friendly and biodegradable (Isman, 2006). Thus, pesticides based on plant essential oils are an attractive alternative to control large numbers of insect pests. Essential oils from plants and their components are a potent source of botanical pesticides, which are selective and have little or no harmful effect on mammals and organisms as an eco-friendly alternative to traditional synthetic insecticides. (Liang et al., 2013; Utono and Gibson, 2015; Koutsaviti et al., 2017). Here, contact and fumigant toxicities of the essential oil of Cilantro, *Eryngium foetidum* L., from fresh leaves were determined, and repellent activity against the adult stage of *T. castaneum* was evaluated. Results will provide useful information for further development of new natural insecticides for stored-product insect pests.

Results

Contact toxicity

Contact toxicity of *E. foetidum* EO was compared based on LD_{50} values (Table 1). Six different doses of *E. foetidum* EO were used and their activities after 24, 48 and 72 h were assessed. *E. foetidum* EO showed highest contact toxicity to adults of *T. castaneum* within 72 h after treatment, with LD_{50} of 31.94 µL/mg adult compared with 55.55 and 42.40 µL/mg adult at 24 and 48 h, respectively.

Considerable differences in adult mortality of *T. castaneum* to *E. foetidum* EO were observed for different doses and exposure times. Mortality increased with rising doses from 5 to 25 µL/mg adult and exposure time 24 to 120 h, with highest significant difference ($p \leq 0.5$). Insects exposed to essential oil after treatment at 120 h showed mortality values ranging 30.00-62.50% (Table 2). At doses of 20 and 25 µL/mg adult, *E. foetidum* EO exhibited moderate insecticidal activity at 50% and 62.5%, respectively after 120 h.

Fumigant toxicity

Table 3 shows results of vapor phase toxicity bioassay related to the fumigant activity of *E. foetidum* EO against adults of *T. castaneum*. *E. foetidum* EO was toxic to *T. castaneum* calculated in terms of concentration (LC_{50}) within 72 h. Six different concentrations of *E. foetidum* EO were used and their activities after 24, 48 and 72 h were evaluated. Probit analysis showed that adults of *T. castaneum* were susceptible to *E. foetidum* EO, with highest fumigant toxicity within 72 h after treatment with LC_{50} of 13.03 µL/L air compared to 58.04 and 17.96 µL/L air at 24 and 48 h, respectively. Significant

Table 1. Contact toxicity (LD_{50}) of *Eryngium foetidum* EO against *Tribolium castaneum* after treatment at 24, 48 and 72 h

Time (h)	n	LD_{50} (µL/mg adult)	95% Fiducial limits	Linear equation y = ax + b	r^2 value
24	240	55.55	31.50-56.15	y = 0.9x + 0.0	0.9529
48	240	42.40	27.58-46.34	y = 1.1286x + 2.1429	0.9536
72	240	31.94	22.81-45.99	y = 1.3714x + 6.1905	0.8263

n is the number of the tested insect population.
r^2 is the correlation coefficient.
LD_{50} (50% lethal dose) is the amount of substance required to kill half of the members of a tested insect population after a specified test duration period, expressed as µL/mg adult.

Table 2. Adult mortality (%) of *Tribolium castaneum* after contact toxicity testing by topical application with six doses of *Eryngium foetidum* EO at 24, 48, 72, 96 and 120

Dose (µL/mg adult)	Adult mortality **(%)** of *T. castaneum*				
	24 h	48 h	72 h	96 h	120 h
0	0.00±0.00 d	0.00±0.00 c	0.00±0.00 c	0.00±0.00 c	0.00±0.00 d
5	2.50±5.00 cd	7.50±5.00 c	12.50±9.57 b	22.50±9.57 b	30.00±8.16 c
10	10.00±0.00 bc	17.50±5.00 b	30.00±8.16 a	35.00±9.57 ab	40.00±8.16 bc
15	15.00±10.00 ab	20.00±8.16 b	30.00±8.16 a	37.50±15.00 ab	40.00±11.55 bc
20	20.00±8.16 a	22.50±5.00 ab	30.00±8.16 a	42.50±15.0 a	50.00±8.16 ab
25	20.00±8.16 a	30.00±8.16 a	37.50±9.57 a	50.00±8.16 a	62.50±12.58 a
F-test	**	**	**	**	**

** represents significant difference at p≤0.01.
Means within the same column followed by the same letter are not significantly different (LSD: p>0.05).

Table 3. Fumigation toxicity (LC_{50}) of *Eryngium foetidum* EO against *Tribolium castaneum* after treatment at 24, 48 and 72 h

Time (h)	n	LC_{50} (µL/L air)	95% Fiducial limits	Linear equation y = ax + b	r^2 value
24	240	58.04	37.16-75.56	y = 0.8122x + 2.8571	0.93
48	240	17.96	9.21-28.15	y = 1.6382x + 20.571	0.72
72	240	13.03	2.75-25.06	y = 1.6993x + 27.857	0.60

n is the number of the tested insect population.
r^2 is the correlation coefficient.
LC_{50} (50% lethal concentration) is the amount of substance required to kill half of the members of a tested insect population after a specified test duration period expressed as µL/L air.

Table 4. Adult mortality (%) of *Tribolium castaneum* after fumigant toxicity testing by vapor phase for six concentrations of *Eryngium foetidum* EO at 24, 48 and 72 h

Conc. (µL/L air)	Adult mortality **(%)** of *T. castaneum*		
	24 h	48 h	72 h
0	0.00±0.00 d	0.00±0.00 f	0.00±0.00 e
2	2.50±5.00 cd	25.00±5.77 e	32.50±12.58 d
4	10.00±8.16 bc	35.00±5.77 d	45.00±10.00 c
8	10.00±8.16 bc	45.00±5.77 c	60.00±8.16 b
16	17.50±5.00 b	55.00±5.77 b	62.50±5.00 ab
32	27.50±5.00 a	65.00±5.77 a	72.50±5.00 a
F-test	**	**	**

** represents significant difference at p≤0.01.
Means within the same column followed by the same letter are not significantly different (LSD: p>0.05).

Table 5. Adult repellent (%) of *Tribolium castaneum* after contact testing with five concentrations of *Eryngium foetidum* EO within 8 h

Conc. (µL/L air)	Adult repellent (%) of *T. castaneum*			
	1 h	2 h	3 h	4 h
2.5	40.00±14.14	67.50±5.00 bc	67.50±12.58 bc	55.00±12.91 c
5	47.50±15.00	57.50±15.00 c	67.50±17.08 bc	72.50±9.57 b
7.5	40.00±14.14	85.00±5.77 abc	85.00±5.77 ab	90.00±8.16 a
10	57.50±27.54	87.50±5.00 ab	87.50±5.00 ab	92.50±5.00 a
12.5	80.00±14.14	92.50±5.00 a	97.50±5.00 a	95.00±5.77 a
F-test	ns	*	**	**
Conc. (µL/L air)	Adult repellent (%) of *T. castaneum*			
	5 h	6 h	7 h	8 h
2.5	65.00±12.91 b	62.50±18.93 b	70.00±14.14 bc	70.00±18.26 ab
5	85.00±5.77 a	85.00±5.77 a	85.00±5.77 ab	92.50±5.00 a
7.5	90.00±11.55 a	87.50±9.57 a	95.00±5.77 a	97.50±5.00 a
10	90.00±8.16 a	85.00±10.00 a	90.00±14.14 a	97.50±5.00 a
12.5	97.50±5.00 a	95.00±5.77 a	100.00±0.00 a	100.00±0.00 a
F-test	**	**	**	*

*represents significant difference at p≤0.05. ** represents significant difference at p≤0.01. Means within the same column followed by the same letter are not significantly different (LSD: p>0.05).

differences in adult mortality of *T. castaneum* to *E. foetidum* EO were found at different concentrations and exposure times. *E. foetidum* EO showed adult mortality of 72.5% against *T. castaneum* compared with the control (acetone alone) and results showed significant differences (Table 4).

Repellent activity

Results showed that repellent activity of *E. foetidum* EO increased with rising concentrations from 2.5 to 12.5 µL/L air and exposure times from 1 to 8 h. Repellent percentage for 12.5 µL/L air of *E. foetidum* EO after treatment at 7 and 8 h showed 100% adult mortality with the highest significant difference (p≤0.01) (Table 5). However, no significant differences were found when comparing concentrations of 5, 7.5 and 10 µL/L air at 92.50-97.50% of adult repellent.

Discussion

Essential oil of *E. foetidum* from fresh leaves showed high contact toxicity (LD_{50}) on adults of *T. castaneum*. *E. foetidum* belongs to the Apiaceae family, which consists of spicy or aromatic plants containing essential oils in different plant parts. Some species of this family are also known to be good sources of essential oils with insecticidal properties (Ebadollahi, 2013; Zarshenas et al., 2014). Liu et al. (2011) reported on the toxicity of *Ostericum sieboldii* EO, belonging to the Apiaceae. The plant exhibited severe contact toxicity on *T. castaneum* adults with an LD_{50} value of 8.47 µg/adult. *E. foetidum* EO was also found to be effective at killing and eliminating *T. castaneum*. Essential oils are composed of abundant secondary substances in aromatic families such as Lamiaceae and Apiaceae (Isman, 2006); they contain many compounds including monoterpenes and sesquiterpenes. Tong and Coats (2010) reported that the insecticidal activity of many plants containing essential oils could be attributed to monoterpenoids that were also reported to act as fumigants and contact toxicants on various insect pests by Rice and Coats (1994), with insecticidal activity reported by Lopez et al. (2008) and Islam et al. (2009). Ebadollahi (2011) showed that longer exposure time and higher concentration of essential oils increased insect mortality rate. This result concurred with Amini et al. (2018) who reported that *Pimpinella anisum* L. (Apiaceae) gave toxicity (LC_{50}) of *T. castaneum* at 24 h for 43.75 µL/L air, similar to toxicity (LC_{50}) of 58.04 µL/L air at 24 h reported by Liu et al. (2011) for *Ostericum sieboldii* (Apiaceae) essential oil. The plant exhibited severe fumigant toxicity with *T. castaneum* adults and gave LC_{50} value of 20.92 mg/L, contradicting Khani and Rahdari (2012) who reported the toxicity of *Coriandrum sativum* L. (Apiaceae) essential oil. This plant showed reduced fumigation toxicity to adults of *T. castaneum*, with LC_{50} value of 318.02 µL/L air at 24 h. Essential oil of *E. foetidum* from fresh leaves was effective against adults of *T. castaneum*. Lee et al. (2017) reported on the essential oil of *Anethum graveolens* L., a plant belonging to the Apiaceae family as an effective insect repellent. Plants from the families Annonaceae, Asteraceae, Apiaceae, Chenopodiaceae, Cupressaceae, Lauraceae, Lamiaceae, Meliaceae, Myrtaceae, Poaceae, Piperaceae, Rutaceae, Verbenaceae and Zingiberaceae are also sources of botanical insecticides (Isman, 1995). Our results showed that essential oils from medicinal plants, especially those of the Apiaceae family, are effective in combating adults of *T. castaneum*.

Materials and Methods

Insect rearing

Adults of the red flour beetle *T. castaneum* were obtained from infested seeds stored in Maha Sarakham Province, Thailand. The insects were cultured following Wanna and Satongrod (2020) by placing 15 pairs (30 adults) on wheat flour mixed with wheat and yeast (13:1 w/w) in a plastic bottle (diameter 23 cm, height 30 cm), covered with a fine mesh cloth for ventilation. The insects were maintained at 30±5°C and 70±5% relative humidity at a 16:8 h (light: dark) photoperiod for the development of progeny. Adult insects emerging after 7 days were used for bioassay tests. All experiments were conducted under the same environmental conditions.

Extraction of essential oil

Essential oil was extracted from fresh leaves of *Eryngium foetidum* L. bought from a local market in Muang district, Maha Sarakham Province and kept in a refrigerator. Fresh leaves of *E. foetidum* were washed and air-dried in the shade, then sliced into small pieces using a sharp blade and chopping board. A Clevenger-type apparatus was used to extract the essential oil from an air-dried sample of fresh leaves of *E. foetidum* (300 g) mixed with 1,000 mL of distilled water in a 2,000 mL round glass bottle. The bottle was distilled at 100-150°C for 6 h at the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University. The essential oil distillate of *E. foetidum* was purified using a centrifuge at a spin speed of 8,000 rpm for 10 min, and kept into an amber bottle with the lid closed in the dark at 4°C until required for further bioassays.

Contact toxicity

Contact toxicity of *Eryngium foetidum* EO against *T. castaneum* was assessed using the topical application test, with six doses as 0 (control), 5, 10, 15, 20 and 25 µL/mg adult under 30±5°C and 70±5% relative humidity at a 16:8 h (light: dark) photoperiod. Dilutions of *E. foetidum* EO were prepared in acetone. Controls were determined using acetone alone, with each concentration replicated independently four times. *T. castaneum* adults were placed in a freezer for 1 min to reduce their activity and allow topical treatment applications. Aliquots of 0.5 mL of the dilutions were applied topically to the dorsal thorax of *T. castaneum* using a microsyringe. Each treated insect was then transferred to a Petri dish (10 insects per Petri dish) and kept in an incubator. Adult mortality of *T. castaneum* was recorded after 24, 48, 72, 96 and 120 h. The insects were considered to be dead if no leg or antennal movements were detected.

Fumigation toxicity

Fumigant toxicity of *E. foetidum* EO was tested with the vapor phase test as previously described (Wanna and Krasaetep, 2019). Whatman (no.1) filter paper strips (length 1.5 cm x width 5 cm) were impregnated with 100 µL of 0 (control), 2, 4, 8, 16 and 32 µL/L air dilution of *E. foetidum* EO as prepared earlier. The solvent was allowed to evaporate for 2 min at room temperature. Filter paper strips were hung in glass vials (diameter 2.5 cm x height 5 cm) from the center of a screw cap fumigation bottle (diameter 5.5 cm x height 10.5 cm) to avoid contact between the insects and the paper strip. Ten *T. castaneum* adults were transferred to each fumigation bottle and the cap was screwed tightly shut and kept at 30±5°C and 70±5% relative humidity at a 16:8 h (light: dark) photoperiod. Control sets included insects fumigated with acetone alone. Four replicates were carried out for all treatments and controls. Adult mortality of *T. castaneum* was recorded after 24, 48 and 72 h. When no leg or antennal movements were observed, the insects were considered to be dead.

Repellent activity

Repellent activity of *E. foetidum* EO was assessed for adults of *T. castaneum* by the impregnated paper test. Dilutions of *E. foetidum* EO (2.5, 5, 7.5, 10.0 and 12.5 µL/L air) were prepared using acetone as the solvent. Each replicate was conducted in Petri dishes (diameter 90 mm × height 20 mm) covered with Whatman filter papers (diameter 9 cm) with one half treated with *E. foetidum* EO and the other half treated with acetone alone as a control. Each half of the filter paper disks was treated separately with 300 µL of liquid using a micropipette (100 µL). Both treated and control half disks were air-dried under a fan to evaporate the solvent completely within 2 min at room temperature. The halves were then affixed at the center of the Petri dishes using adhesive tape. Twenty *T. castaneum* adults were released at the center of the paper disk, then covered with a Petri dish and kept at 30±5°C and 70±5% relative humidity at a 16:8 h (light: dark) photoperiod. All treatments were replicated four times on different days under the same rearing conditions. The number of *T. castaneum* adults present in the control and treated areas was recorded after testing a 1, 2, 3, 4, 5, 6, 7 and 8 h.

Statistical analysis

Data were adjusted for control mortality according to Abbott's formula (Abbott, 1925), and mortality in the control ranged between 5 and 20%. Mortality data and Percentages of repellent on the treated side of the paper disk were analyzed using the F-test statistic by one-way analysis of variance, and mean values were compared using the least significant difference test (LSD) at 0.05 probability level ($p \leq 0.05$). The LC_{50} values were calculated using Probit analysis.

Conclusions

Essential oil of *E. foetidum* from fresh leaves showed contact and fumigant activities against *T. castaneum* and reduced the risks associated with using synthetic insecticides for stored grain protection. High repellent activity against *T. castaneum* was demonstrated by *Eryngium foetidum* EO at a concentration of 12.5 µL/L air, with a notable biological effect on migratory ability. Our data supported previous research outcomes on insecticidal activity of essential oils and their possible use as natural pesticides. The mode of action of *E. foetidum* EO is of special interest, and further studies should focus on its biological activity against economically harmful species of insect, and effects on other non-target organisms as relevant for the development of insect pest control. Essential oil from *E. foetidum* shows promise as an interesting alternative to conventional chemical control strategies.

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