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CONTENTS

Long term efficacy of seven essential oils against *Sitophilus oryzae* (Linnaeus), *Rhizopertha dominica* (Fabricius) and *Tribolium castaneum* (Herbst)

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ABSTRACT: Experiments were conducted to study the long-term bio-efficacy and fumigant toxicity of seven plants essential oils extracted from *Mentha cardiaca*, *Tanacetum cinerariifolium*, *Ocimum basilicum*, *Lippia alba, Ferula assa-foetida, Salvia officinalis* and *Lavandula angustifolia* against *Rhyzopertha dominica* (Fabricius), *Sitophilus oryzae* (Linnaeus) and *Tribolium castaneum* (Herbst.) at 0.1, 0.2, 0.3, and 0.4 per cent (v/w) concentration. The essential oils of *M. cardiaca* and *O. basilicum* completely checked the progeny production of *S. oryzae* for 180 days at all four concentrations in both the preliminary as well as confirmatory tests while such pronounced effect was exhibited by *T. cinerariifolium* at 0.2-0.4 per cent; *L. angustifolia* at 0.3-0.4 per cent and *L. alba* at 0.4 per cent only. In case of *R. dominica,* the oils of *M. cardiaca, T. cinerariifolium, O. basilicum* and *F. assa-foetida* completely checked the F1 progeny for 228 days in preliminary and 220 days in confirmatory test at all four concentrations while complete inhibition was achieved by *L. angustifolia* at 0.2-0.4 per cent and *L. alba* and *S. officinalis* at 0.3-0.4 per cent. The essential oil of *O. basilicum* completely checked the reproduction of *T. castaneum* at 0.1-0.4 per cent for 90 days while such high efficacy was shown by *M. cardiaca* at 0.2-0.4 per cent and *T. cinerariifolium* and *L. angustifolia* at 0.4 per cent. The study revealed that essential oils are highly effective against all the major insect pests of stored grains and they can be used for preventing the post-harvest infestation of stored grains.

Key words: Bio-efficacy, essential oils, fumigant toxicity, *Rhyzopertha dominica*, *Sitophilus oryzae, Tribolium castaneum*

Stored grain insects such as Lesser grain borer (*Rhyzopertha dominica*), Rice weevil (*Sitophilus oryzae*) and Rust- red flour beetle (*Tribolium castaneum*) are the serious pest of stored grains and their products in several countries. The species of *Sitophilus* and *Rhyzopertha* are classified as primary pests while *Tribolium* is a secondary pest of stored products. In most of the countries several types of insecticide and fumigants are being used for the protection of stored grain and their products from insect infestation, however, injudicious use of such hazardous chemicals is causing severe health hazards and environmental contamination due to which some products are being taken out from the market. Extensive research is also being done in several countries to find out the safe alternatives for which scientists are exploring the potential of several natural resources which have been used in past as traditional grain protectants. Most of the traditional methods are based on use of naturally available materials of plant origin which work as ovicidal, repellent, antifeedant, chemo sterilant, toxic to insects, disinfectant or protectant(Nawrot and Harmatha, 1994; Isman, 2006; Lee *et al*., 2020). The toxicity may be by contact, ingestion or through fumigant action. Much emphasis has been given on volatile and non-volatile materials of plant origin which are easily available in most of the ecosystem throughout the world. Some attempts have also been made to describe and enlist such natural resources useful for grain protection (Grainge and Ahmed, 1988). Now a days special attention is being given to use of essential oils extracted from various aromatic and medicinal plants because these essential oils are highly effective against insect pests, volatile, biodegradable and have good diffusion and penetration power (Shaaya *et al.,* 1990; Rajendran and Sriranjini, 2008; Tewari and Tiwari, 2008; Geetanjly *et al.*, 2016; Kumar and Tiwari, 2017a; 2017b; Kumar and Tiwari, 2018a; Kumar and Tiwari, 2018b; Joshi and Tiwari, 2019; Sharma and Tiwari, 2021a; Geetanjly and Tiwari, 2021; Tewari and Tiwari, 2021a; 2021b;2021c). These essential oils are also capable to protect grain for longer duration (Tewari and Tiwari, 2021d). Focus on the vapor or fumigant toxicity of essential oils of plants and their constituents has sharpened since the 1980s. There are many reviews dealing with the use of plant products against insect pests of stored products (Adler *et al*., 2000; Weaver and Subramanyam, 2000; Isman, 2006), specifically on essential oils (Singh and Upadhyay, 1993; Regnault-Roger,

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1997) and others only on monoterpenoids (Coats *et al*., 1991). Plant essential oils are produced commercially from several botanical sources, Apiaceae (Umbeliferae), Araceae, Asteraceae (Compositae), Brassicaceae (Cruciferae) Chenopodiaceae, Cupressaceae, Graminaceae, Lamiaceae (Labiatae), Lauraceae, Liliaceae, Myrtaceae, Pinaceae, Rutaceae and Zingiberaceae. The essential oil of a plant may contain hundreds of different constituents but certain components are present in larger quantities. For example, 1,8-cineole is predominant in the essential oil of *Eucalyptus* spp., linalool in *Ocimum* spp., eugenol in clove oil (*Syzygium aromaticum*), thymol in garden thyme (*Thymus vulgaris*) and menthol in various species of mint (*Mentha* species), limonene in *Citrus* spp., myrcene in *Curcuma longa*, carvone in *Carum carvi*, asarone in *Acorus calamus* and glucosinolates in plants belonging to Brassicaceae, cyanohydrins in *Manihot esculenta*, thiosulfinates in *Allium spp*., methyl salicylate in *Securidaca longipedunculata* and carvacrol as well as β-thujaplicine in *Thujopsis dolabrata*. (Isman, 1999; Rajendran and Sriranjini, 2008; Gangwar and Tiwari, 2017; Sharma and Tiwari, 2021b).

The review indicates that several attempts have been made to study the fumigant toxicity of various essential oils against insect pests of stored grain and many oils have been found effective against storage insects. However, we do not have much information on long-term bio-efficacy of different oils due to which it is not feasible to use them in grain protection. Therefore, present investigation was undertaken to study the long-term efficacy of seven essential oils against *S. oryzae, R. dominica* and *T. castaneum* which are major pests of stored grain.

MATERIALS AND METHODS

The experiments were conducted in Post-Harvest Entomology Laboratory of Department of Entomology, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar.

Culture of Insects

Pure culture of test insects was developed in the control room maintained at 27°C±1 temperature and 70±5% relative humidity. Plastic jars of about 1.0 kg capacity were used for rearing purpose. At the center of the lid a hole of 1.8 cm diameter was made and covered with 30 mesh copper wire net to facilitate aeration in the jar. The adults of *R. dominica* and *S. oryzae* were reared on the grain of wheat variety PBW-343 while *T. castaneum* was cultured on its flour fortified with 5 per cent yeast powder. Before use, grains were disinfected in the oven at 60° C for 12 hrs. After disinfestation the moisture content of the grain was measured and raised to 13.5 per cent by mixing water in the grain. The quantity of water required to raise the moisture content was calculated by using following formula as described by Pixton (1967).

$$\text{Quantity of water to be added} = \frac{W_1(M_2-M_1)}{100-M_2}$$

Where,

W_1 = Initial weight of grains
M_1 = Initial moisture content
M_2 = Final moisture content

After mixing the water in grain, it was kept in closed polythene bags for a week so that moisture content of grain could equilibrate. The grain was then filled in plastic jar and 100 adults were released in each jar after which it was kept in control room. To prepare the culture medium of *T. castaneum*, wheat grain was ground to a fine powder and yeast powder was mixed in it at the rate of 5 per cent. The medium was filled in plastic jars and adults were released in it. First generation adults (0-7 days old) were used for experimental purpose.

Procurement of Oils

The experimental oils were procured from the Medicinal and Aromatic Plants Research and Development Centre, Pantnagar, Central Institute of Medicinal and Aromatic Plants, Field Station, Nagla and Central Institute of Medicinal and Aromatic Plants, Lucknow. The common and scientific name of plants, the oils of which were used in the experiment is given in Table 1.

Preparation of Grain

All fumigation experiments on *R. dominica, S. oryzae*, *T. castaneum,* were conducted on untreated graded seed of wheat variety PBW-343. Before use, the grains were disinfested by keeping them in the oven at 60^0C for 12 hrs. After disinfestation the moisture content of grain was measured and raised to 13.5 per cent by adding water in the required quantity to the grain. To ensure the even distribution of water, the grain was spread on a platform and water was sprayed on it using hand sprayer. The grain was then mixed thoroughly and closed in polythene bags for a week for equilibration of moisture content of grain. The grain (50g) was then filled in 100ml capacity plastic vials to perform experiment.

Details of Experiment Conducted

The long-term efficacy of essential oils was studied at 0.1, 0.2, 0.3 and 0.4 per cent (v/w) against adults of R. *dominica, S. oryzae* and *T. castaneum* (Table-1). The

Table 1: Common and scientific name of plants the essential oil of which was used to study fumigant toxicity

Sl. No.	Scientific name	Common name	Family	Concentrations % (v/w)
1.	*Ferula assafoetida* Linn.	Ferula	Apiaceae	0.1, 0.2, 0.3, 0.4
2.	*Lavendula angustifolia*	Lavender	Lamiaceae	0.1, 0.2, 0.3, 0.4
3.	*Lippia alba*	Bushymatgrass	Verbenaceae	0.1, 0.2, 0.3, 0.4
4.	*Mentha cardiaca* (S.F. Gray) Bak.)	Scotch spearmint	Lamiaceae	0.1, 0.2, 0.3, 0.4
5.	*Ocimum bacilicum* Linn.	Tulsi	Lamiaceae	0.1, 0.2, 0.3, 0.4
6.	*Salvia officinalis* Linn.	Common sage	Lamiaceae	0.1, 0.2, 0.3, 0.4
7.	*Tanacetum cinerariifolium* Sch.Bip	Tanacetum	Asteraceae	0.1, 0.2, 0.3, 0.4

Table 2: Number of adults of *S. oryzae* emerged in grain treated with different essential oils in preliminary and confirmatory test

Essential oils	Dose (%) v/w	Preliminary test		Confirmatory test	
		Number of adults emerged	Days after fumigation	Number of adults emerged	Days after fumigation
M. cardiaca	0.1	0.0±0.0	180	0.0±0.0	180
M. cardiaca	0.2	0.0±0.0	180	0.0±0.0	180
M. cardiaca	0.3	0.0±0.0	180	0.0±0.0	180
M. cardiaca	0.4	0.0±0.0	180	0.0±0.0	180
T. cinerariifolium	0.1	0.0±0.0	180	164.6±164.7	180
T. cinerariifolium	0.2	0.0±0.0	180	0.0±0.0	180
T. cinerariifolium	0.3	0.0±0.0	180	0.0±0.0	180
T. cinerariifolium	0.4	0.0±0.0	180	0.0±0.0	180
O. bacilicum	0.1	0.0±0.0	180	0.0±0.0	180
O. bacilicum	0.2	0.0±0.0	180	0.0±0.0	180
O. bacilicum	0.3	0.0±0.0	180	0.0±0.0	180
O. bacilicum	0.4	0.0±0.0	180	0.0±0.0	180
L. alba	0.1	0.0±0.0	180	538.3±123.4	120
L. alba	0.2	0.0±0.0	180	410.7±85.51	180
L. alba	0.3	144.3±144.3	180	236.0±119.1	180
L. alba	0.4	0.00±0.00	180	0.0±0.0	180
S. officinalis	0.1	474.3±241.9	180	478.0±212.6	120
S. officinalis	0.2	0.0±0.0	180	234.3±234.3	120
S. officinalis	0.3	0.0±0.0	180	407.7±205.3	120
S. officinalis	0.4	0.0±0.0	180	288.7±113.2	180
F. assafoetida	0.1	577.0±205.1	180	234.7±142.8	180
F. assafoetida	0.2	122.7±122.7	180	37.0±30.3	180
F. assafoetida	0.3	0.0±0.0	180	0.0±0.0	180
F. assafoetida	0.4	33.0±16.6	180	0.0±0.0	180
L. angustifolia	0.1	409.7±207.7	180	840.0±30.4	120
L. angustifolia	0.2	0.0±0.0	180	180.3±108.3	180
L. angustifolia	0.3	0.0±0.0	180	0.0±0.0	80
L. angustifolia	0.4	0.0±0.0	180	0.0±0.0	80
Control	-	852.7±34.4	150	821.7±28.3	80

experiment was conducted under controlled conditions at 27+1^{0}C temperature and 70+ 5 per cent relative humidity. Fifty-gram wheat grains of variety PBW-343 (moisture content 13.5 per cent) was filled in each plastic vial. Each treatment was replicated 3 times. Untreated grain was used as control. Different set was prepared for each insect. Ten adults of *R. dominica, S. oryzae* or *T. castaneum* (0-7 days old) were released in one vial. After 24 hrs. of releasing the insects measured quantity of oil was poured on the absorbing mat, which was then placed inside the vial between the grains. Screw cap of vials was then tightly closed.

Insects were then allowed to feed and breed on the treated grain. The insects emerging in the vial were counted after appearance of very high symptoms of infestation in untreated control. The experiments were terminated after 180, 220-228 and 90 days in case of *S. oryzae*, *R. dominica* and *T. castaneum*, respectively, and observations were recorded on number of adults emerged in each vial. All

Table 3: Number of adults of *R. dominica* emerged in grain treated with different essential oils in preliminary and confirmatory test

Essential oils	Dose (%) v/w	Preliminary test		Confirmatory test	
		Number of adults emerged	Days after fumigation	Number of adults emerged	Days after fumigation
M. cardiaca	0.1	0.0±0.0	228	0.0±0.0	220
M. cardiaca	0.2	0.0±0.0	228	0.0±0.0	220
M. cardiaca	0.3	0.0±0.0	228	0.0±0.0	220
M. cardiaca	0.4	0.0±0.0	228	0.0±0.0	220
T. cinerariifolium	0.1	0.0±0.0	228	0.0±0.0	220
T. cinerariifolium	0.2	0.0±0.0	228	0.0±0.0	220
T. cinerariifolium	0.3	0.0±0.0	228	0.0±0.0	220
T. cinerariifolium	0.4	0.0±0.0	228	0.0±0.0	220
O. bacilicum	0.1	0.0±0.0	228	0.0±0.0	220
O. bacilicum	0.2	0.0±0.0	228	0.0±0.0	220
O. bacilicum	0.3	0.0±0.0	228	0.0±0.0	220
O. bacilicum	0.4	0.25±0.33	228	0.0±0.0	220
L. alba	0.1	478.8±335.2	228	227.5±271	220
L. alba	0.2	229.8±305.3	228	0.0±0.0	220
L. alba	0.3	0.0±0.0	228	0.0±0.0	220
L. alba	0.4	0.0±0.0	228	0.0±0.0	220
S. officinalis	0.1	692.5±87.8	228	333.0±244.9	220
S. officinalis	0.2	102.8±34.6	228	42.3±56.3	220
S. officinalis	0.3	0.0±0.0	228	0.0±0.0	220
S. officinalis	0.4	0.0±0.0	228	0.0±0.0	220
F. assafoetida	0.1	0.0±0.0	228	0.0±0.0	220
F. assafoetida	0.2	0.0±0.0	228	0.0±0.0	220
F. assafoetida	0.3	0.0±0.0	228	0.0±0.0	220
F. assafoetida	0.4	0.0±0.0	228	0.0±0.0	220
L. angustifolia	0.1	410.0±277.8	228	0.0±0.0	220
L. angustifolia	0.2	0.0±0.0	228	0.0±0.0	220
L. angustifolia	0.3	0.0±0.0	228	0.0±0.0	220
L. angustifolia	0.4	0.0±0.0	228	0.0±0.0	220
Control	_	807.5±410.5	228	806.5±236.1	220

the experiments were conducted twice to confirm the bio-efficacy of these seven essential oils. The first test was designated as Preliminary Test while second was named as Confirmatory Test. The conclusion about the bio-efficacy of essential oil was drawn on the performance of oil in both the tests and oils showing complete inhibition of progeny in both the rests were rated as highly effective.

RESULTS AND DISCUSSION

The fumigant toxicity of some essential oils against *S. oryzae* is presented in Table 2 which indicates that the oil of *M. cardiaca* and *O. basilicum* were highly effective against *S. oryzae* at 0.1-0.4 per cent for 180 days as no adults emerged in the grain treated with these oils in both preliminary and confirmatory test. The essential oil of *T. cinerariifolium* was also effective against this insect at 0.2-0.4 per cent in both the tests conducted for 180 days. The performance of *L. alba* oil was not consistent in both the tests at 0.1-0.3 per cent. However, at 0.4 per cent the essential oil of *L. alba* completely checked the progeny production of *S. oryzae* for 180 days. The essential oil of *S. officinalis* was effective against this insect at 0.2-0.4 per cent in preliminary test. However, in the confirmatory test it failed to check the progeny production at all doses due to which this oil was not classified as effective against *S. oryzae*. The essential oil of *F. assa-foetida* was effective against this insect at 0.3-0.4 per cent while *L. angustifolia* oil completely checked the progeny production of this insect at 0.3-0.4 per cent. Rozman *et al.* (2007) reported that 1,8-cineole, camphor, eugenol, linalool, carvacrol, thymol, borneol, bornyl acetate and linalyl acetate occur naturally in the essential oils of the aromatic plant oil of *L. angustifolia* was highly effective against *S. oryzae*.

The bio-efficacy of the above-mentioned essential oil against *R. dominica* is presented in Table 3 which indicates that the oil of *M. cardiaca*, *T. cinerariifolium*, *O. basilicum*

Table 4: Number of adults of *T. castaneum* emerged in grain treated with different essential oils in preliminary and confirmatory test

Essential oils	Dose (%) v/w	Preliminary test		Confirmatory test	
		Number of adults emerged	Days after fumigation	Number of adults emerged	Days after fumigation
M. cardiaca	0.1	0.0±0.0	90	10.7±3.7	90
M. cardiaca	0.2	0.0±0.0	90	0.0±0.0	90
M. cardiaca	0.3	0.0±0.0	90	0.0±0.0	90
M. cardiaca	0.4	0.0±0.0	90	0.0±0.0	90
T. cinerariifolium	0.1	35.3±6.2	90	36.3±6.7	90
T. cinerariifolium	0.2	18.7±9.4	90	5.0±4.0	90
T. cinerariifolium	0.3	3.3±3.3	90	19.0±4.0	90
T. cinerariifolium	0.4	0.0±0.0	90	0.0±0.0	90
O. basilicum	0.1	0.0±0.0	90	0.0±0.0	90
O. basilicum	0.2	0.0±0.0	90	0.0±0.0	90
O. basilicum	0.3	0.0±0.0	90	0.0±0.0	90
O. basilicum	0.4	0.0±0.0	90	0.0±0.0	90
L. alba	0.1	33.3±2.9	90	25.3±5.8	90
L. alba	0.2	32.7±2.0	90	44.3±5.4	90
L. alba	0.3	24.3±1.8	90	11.7±8.7	90
L. alba	0.4	29±1.53	90	22.7±2.8	90
S. officinalis	0.1	27.0±1.7	90	25.7±17.2	90
S. officinalis	0.2	32.3±3.5	90	21.7±5.2	90
S. officinalis	0.3	11±5.7	90	16.7±1.5	90
S. officinalis	0.4	4.3±4.3	90	8.0±4.5	90
F. assafoetida	0.1	41.7±1.7	90	25.3±10.7	90
F. assafoetida	0.2	31.0±4.6	90	38.7±8.3	90
F. assafoetida	0.3	19±10.9	90	21.7±6.9	90
F. assafoetida	0.4	27.0±1.0	90	15.3±7.8	90
L. angustifolia	0.1	27.0±6.1	90	36±18.1	90
L. angustifolia	0.2	24.0±4.9	90	14.3±6.4	90
L. angustifolia	0.3	0.0±0.0	90	5.7±2.4	90
L. angustifolia	0.4	0.0±0.0	90	0.0±0.0	90
Control	_	70.3±12.5	90	41.3±11.6	90

and *F. assa-foetida* were highly effective against this insect for 228 days as they completely checked the progeny production of this insects in both the studies at 0.1-0.4 per cent. The oil of *L. alba* and *S. officinalis* were effective against *R. dominica* at 0.3-0.4 per cent at which it completely suppressed the progeny of this insect. The *L. angustifolia* oil completely suppressed the breeding of *R. dominica* at 0.2-0.4 per cent in both the studies. The *L. angustifolia* and *S. officinalis* oils were also reported to be highly effective against *R. dominica* (Shaaya *et al*.,1990).

The fumigant toxicity of essential oils against *T. castaneum* is present in Table 4 which indicates that *M. cardiaca* completely suppressed the progeny of this insect at 0.2-0.4 per cent for 90 days in both the studies. The oil of *T. cinerariifolium* was highly effective only at 0.4 per cent while *O. basilicum* oil completely suppressed the F_1 progeny of *T. castaneum* at 0.1-0.4 per cent in both the studies. In another study Lee *et al.* (2021) have also reported that the oil of *T. cinerariifolium* exhibited effective repellency against the adults and larvae of the *T. castaneum*. The oil of *L. alba*, *S. officinalis* and *F. assa-foetida* were not found to be effective against *T. castaneum*. The oil of *L. angustifolia* completely suppressed the progeny of *T. castaneum* at 0.4 per cent while some adult emerged at 0.3 per cent in confirmatory test. Effectiveness of *L. angustifolia* oil against this insect was also observed by Novokmet *et al*. (2002).

CONCLUSION

The present study revealed that the essential oils evaluated in this study were highly effective against insect pests of stored grain, however, their efficacy was influenced by species of insect pests and dose of the oil. The essential oil of *M. cardiaca* was highly effective against all three species of insects at 0.2-0.4 per cent while at lowest

Table 5: Average number of adults of *S. oryzae, R. dominica* and *T. castaneum* emerged in grain treated with different essential oils

Essential oils	Dose (%) v/w	*S. oryzae*	*R. dominica*	*T. castaneum*
M. cardiaca	0.1	0	0	6
M. cardiaca	0.2	0	0	0
M. cardiaca	0.3	0	0	0
M. cardiaca	0.4	0	0	0
T. cinerariifolium	0.1	83	0	36
T. cinerariifolium	0.2	0	0	12
T. cinerariifolium	0.3	0	0	11
T. cinerariifolium	0.4	0	0	0
O. basilicum	0.1	0	0	0
O. basilicum	0.2	0	0	0
O. basilicum	0.3	0	0	0
O. basilicum	0.4	0	0	0
L. alba	0.1	269	354	29
L. alba	0.2	206	115	39
L. alba	0.3	190	0	18
L. alba	0.4	0	0	26
S. officinalis	0.1	476	513	27
S. officinalis	0.2	117	73	27
S. officinalis	0.3	204	0	14
S. officinalis	0.4	145	0	6
F. assafoetida	0.1	406	0	34
F. assafoetida	0.2	80	0	35
F. assafoetida	0.3	0	0	21
F. assafoetida	0.4	17	0	21
L. angustifolia	0.1	625	205	32
L. angustifolia	0.2	90	0	19
L. angustifolia	0.3	0	0	3
L. angustifolia	0.4	0	0	0
Control	_	838	808	56

concentration of 0.1 per cent it showed high efficacy only against *S. oryzae* and *R. dominica*. In case of *T. cinerariifolium*, high efficacy was recorded against all three insects at highest concentration of 0.4 per cent, however, it was also found to be highly effective against *S. oryzae* and *R. dominica* at 0.2-0.3 per cent. At the lowest concentration of 0.1 per cent, this oil was highly effective only against *R. dominica*. The essential oil of *O. basilicum* was highly effective against all three insects at all the concentrations. The oil of *L. alba* showed high efficacy against *R. dominica* and *S. oryzae* at 0.4 per cent, however, it also showed high efficacy against former at 0.3 per cent. The essential oil of *S. officinalis* was not effective against *S. oryzae* and *T. castaneum* at any concentration tested, however, it showed high efficacy against *R. dominica* at 0.3-0.4 per cent. The oil of *F. assa-foetida* was highly effective only against *R. dominica* at 0.1-0.4 per cent. The essential oil of *L. angustifolia* was found to be highly effective against all three insect species at 0.4 per cent. This oil also showed high efficacy against *S. oryzae* at 0.3 per cent and *R. dominica* at 0.2-0.3 per cent. It may be concluded that the essential oils of all seven plants are very useful in protection of grain under storage condition and they may be utilized in formulation of a product highly effective against all the insect species.

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