

Chemical characterization and anti-parasitic property of essential oil of *Coriandrum sativum* leaf against *Trichomonas vaginalis*

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ABSTRACT

Coriandrum sativum has been used in Iranian traditional medicine as an anti-inflammatory, antioxidant, antibacterial, and antifungal agent. The purpose of this study was to evaluate the chemical composition and anti-parasitic property of essential oil of *C. sativum* leaf on trophozoite of *Trichomonas vaginalis*. *C. sativum* was collected from Kermanshah city and essential oil was prepared by the Clevenger device. The essential oil was analyzed by GC/MS. Trophozoite of *T. vaginalis* was cultured in vitro in CPLM medium and the effect of the essential oil on the survival of *T. vaginalis* trophozoite was measured by Neobar slide. This study indicated that Linalool (71.2%) was the most constituent found in *C. sativum* essential oil. Also, the results of anti-parasitic tests demonstrated the concentrations of 0.5, 0.25, 0.125, 0.062, 0.031, 0.015, 0.007, 0.003, and 0.001 g/ml in essential oil and 0.25 g/ml in metronidazole could destroy of *T. vaginalis* trophozoite completely after 420 minutes incubation. The best results were observed at 0.5 and 0.25 g/ml concentrations of essential oil, because these concentrations were able to destroy trophozoite in 90 minutes. Also, 0.001 g/ml concentration of essential oil had the lower anti-parasitic effect than all concentrations against *T. vaginalis* trophozoite. The trophozoite survived at DMSO after 600 minutes. MIC and MLC of *C. sativum* essential oil were 0.015 and 0.031 g/ml concentrations, respectively. In our study, the essential oil of *C. sativum* leaf in several concentrations destroyed *T. vaginalis* trophozoite. It appears that *C. sativum* can be used for the treatment of some *T. vaginalis* infections as an antibiotic.

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Keywords

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INTRODUCTION

Antibiotics as one of the most important drugs in pharmacopeia every country, make the fundamental basis for the prevention, control, and treatment of microbial diseases. However excessive use of antibiotics has led to the production of multi-drug resistant strains [1]. One way to limit the resistance of pathogenic microorganism species to the anti-

biotic is using of plants [2]. Herbal remedies have significant benefits such as fewer side effects, better patient tolerance, and better accessibility [3-5]. Plants as a rich source of ethno medicinal compounds have continued to play a distinguished role in the maintenance of human health against several diseases [6]. Many plants are used for their anti-parasitic prop-

erties [7-9]. Furthermore, the recent development of methods such as essential oil extraction, has increased the interest of herbs researchers to the phytochemical researches [10-13]. Essential oils could be extracted from several parts like roots, stems, leaves, flowers, and fruits. In recent years, interest in essential oils has been incremented for pharmacological studies and it appears that the essential oils have been useful for control and inhibition of animal and human parasitic disease such as *Trichomonas vaginalis* infections [14-16].

Iran has a rich flora that is widely distributed throughout the country, particularly in the west of Iran [17-19]. In Iranian traditional medicine, herbal medicines have been the basis of treatment and cure for *T. vaginalis* diseases. A list of medicinal plants in Iran that are consumed for their anti-trichomoniasis properties including *Artemisia aucheri* Boiss, *Zataria multiflora* Boiss, *Myrtus communis*, *Allium sativum*, *Ferula assa-foetida*, *Lavandula angustifolia*, *Eucalyptus camaldulensis*, *Stachys sylvatica*, *Achillea millefolium*, *Artemisia absinthium*, *Juglans regia*, *Tanacetum parthenium*, *Taxus baccata*, *Viola odorata*, *Pelargonium roseum*, *Verbascum Thapsus*, *Allium Cepa*, *Oliveria Decumbens* Vent, *Muscari neglectum* [20].

One of the most important herbal medicines, which is widely consumed in Iranian traditional medicine for the treatment of parasitic infections is *Coriandrum sativum* or *Coriander* from *Apiales* order, *Apiaceae* family, *Coriandrum* genus [21]. *C. sativum* is one of the edible plants which have generated a lot of interest throughout human history as a medicinal plant. Several extracts of the plant are traditionally used in treating the gastric ulcer, viral, fungal, and bacterial diseases [22]. As far as we know, there is a very little data about the anti-trichomoniasis effect of *C. sativum* essential oil collected from Kermanshah province, west of Iran. Hence, the aim of the present study was assessment chemical composition and anti-parasitic property of *C. sativum* on *T. vaginalis* trophozoite.

MATERIALS AND METHODS

Cultivation of *T. vaginalis* trophozoite

T. vaginalis trophozoite was procured from Iran Pasteur Institute. For the mass cultivation, in sterile conditions, we cultured the parasite samples CPLM (Hi-Media Laboratories PVT Ltd Company) at a temperature of 37 °C. Then, we have added 5 ml of the medium to the CPLM culture medium and have put at a temperature of 22-24 °C. After 72 hours, we review the medium regarding parasite growth and the absence of bacterial and fungal contamination we repeated this every 72 hours until the intended parasite volume was obtained.

Plant sample collection and essential oil extraction

In the tentative study, the plant collected from Kermanshah. The sample was purified from any strange, plants, dust, or any other contaminants. Essential oil from *C. sativum* leaf extracted by hydro-steam distillation using the Clevenger device was collected and stored in vials. Briefly,

100 to 150 g of the plant was introduced in the distillation flask (1L), which was conjunct to a steam generator via a glass conduit and to a condenser to regain the essential oil. This was retrieved in a funnel spout. Aromatic molecules of the essential oil were assoiled from the plant material and evaporated into hot steam. The hot steam forced the plant material to liberate the essential oil without burning the plant material itself. Then, hot steam containing the essential oil was elapsed through a cooling system to compress the steam. The steam was applied for 3h. After settling the recovered mixture, essential oil was withdrawn. The supernatant essential oil was cleaned up through anhydrous Na₂SO₄ to desiccate the yielded essential oil. Then, the essential oil was collected in tightened vials and stored in a refrigerator.

Essential oil of *C. sativum* analyzed by GC-MS, fused silica DB-5 column with 0.25 µm thickness film was used. The oven temperature was kept at 500°C for 10 minutes and then regulated at 50-2800°C for 40 minutes. Helium flow rate was maintained at 2 ml/min, with the split ratio of 1:3. Ionization voltage of GC/MS was run at 70ev. The constituents of essential oil were recognized by GC/MS. NIST standard reference database (AMDIS version 2.70) was used to interpret the mass spectral data.

The effect of essential oil of *C. sativum* on *T. vaginalis* trophozoite

Different concentrations of essential oil of *C. sativum* were prepared the 0.5 g/ml from which nine fold serial dilutions (v/v) (0.5, 0.25, 0.125, 0.062, 0.031, 0.015, 0.007, 0.003, and 0.001 g/ml). Metronidazole (0.25g/ml) was used as a positive control and Dimethyl sulfoxide (DMSO) (Merk, Germany) was used as a negative control. Above doses of *C. sativum* essential oil, metronidazole and DMSO individually added to *T. vaginalis* trophozoite content tubes. All tubes were examined unknowingly with Neobar slide regarding viability and motility from the beginning of cultivation with 15- minute intervals up to 3 hours and then in 4, 5, 6, 7, 8, 9, 10 hours. To determine minimum inhibitory concentrations (MIC), all dilutions were prepared from the essential oil in culture media. Then, 60 µl of the microbial suspension was added to each dilution. Finally, the inoculated tubes were incubated at 37 °C for 24 h. After incubation, the tubes were examined regarding the turbidity caused by inoculated the growth of the parasite. The minimum dilution of the essential oil with no turbidity (lack of growth) was considered MIC. To determine minimum lethal concentrations (MLC), all growth-free tubes were cultured by agar media. The inoculated media were incubated at 37 °C for 24 h. The plates with the minimum concentration of essential oil and no parasitical survival were considered MLC of that concentration of the essential oil. Anti-parasitic effect of the *C. sativum* essential oil was tested five times [23].

Statistical Analysis

The data were analyzed by SPSS-22 software using one-way ANOVA followed by Duncan test. To determine the normality of data, the Kolmogorov-Smirnov test was ap-

plied. Data were reported as Mean±SD, and $p \leq 0.01$ was considered significant.

RESULTS

Chemical composition of *C. sativum* essential oil

Overall, fifteen compounds such as α -pinene, β -pinene, p-cymene, limonene, γ -terpinene, linalool, terpinen-4-ole, decanal, nerol, carvacrol, thymol, neryl acetate, 2e-dodecanal., tetrahydro ionol, n-hexadecane were identified in the essential oil of *C. sativum* using GC/MS (Table 1) and Linalool (71.2%) was the most detected compounds.

In vitro anti-parasitic property of *C. sativum* essential oil against *T. vaginalis* trophozoite

In the review of the effect of essential oil of *C. sativum*

on *T. vaginalis* trophozoite compared with the control groups, it was identified that essential oil of *C. sativum* at all concentrations (especially at 0.5 and 0.25 g/ml concentrations) destroyed trophozoite in a dose-dependent manner. At the beginning of the experiment, the percent of live trophozoite of DMSO, metronidazole and all concentrations of essential oil was 100% (Fig. 1). Over time, the percentage of trophozoite survival in treated groups with several doses of essential oil and metronidazole decreased. The percent of live trophozoite of the essential oil was 0% in the 0.5 and 0.25 g/ml concentrations after 90 minutes of incubation. While the trophozoite alive at other concentrations of essential oil and metronidazole (Fig. 2). The 0.125 and 0.062 g/ml concentrations of essential oil had 0% of percent of live trophozoite after 135 minutes of incubation, but 0.031,

Table 1. Chemical composition of essential oils of *C. sativum*

Compounds	Retention Index.	%
α - Pinene	939	3.1
β - Pinene	978	0.4
p-Cymene	1018	2.3
Limonene	1026	0.3
γ - Terpinene	1052	8.9
Linalool	1085	71.2
Terpinen-4-ole	1164	0.1
Decanal	1180	0.3
Nerol	1212	0.6
Carvacrol	1265	0.3
Thymol	1276	0.2
Neryl acetate	1342	7.2
2E-Dodecanal	1408	3.5
Tetrahydro ionol	1539	0.4
n-Hexadecane	1601	0.4
Total	-	99.2

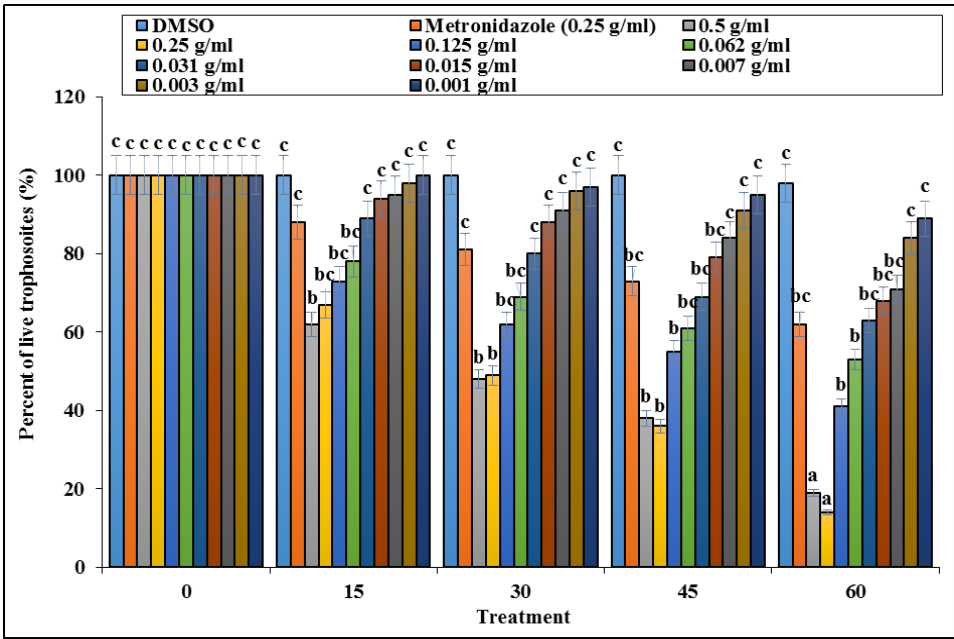


Figure 1. Effect of essential oil of *C. sativum* on the percent of live *T. vaginalis* trophozoites in 0, 15, 30, 45 and 60 minutes. Non-identical letters indicate a significant difference between the groups ($p \leq 0.05$).

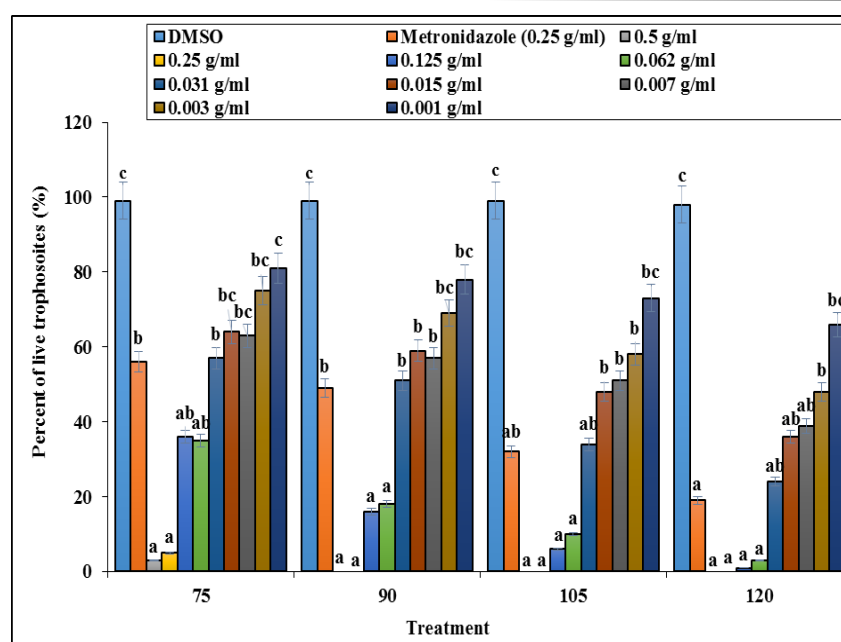


Figure 2. Effect of essential oil of *C. sativum* on the percent of live *T. vaginalis* trophozoites in 75, 90, 105 and 120 minutes. Non-identical letters indicate a significant difference between the groups ($p \leq 0.05$).

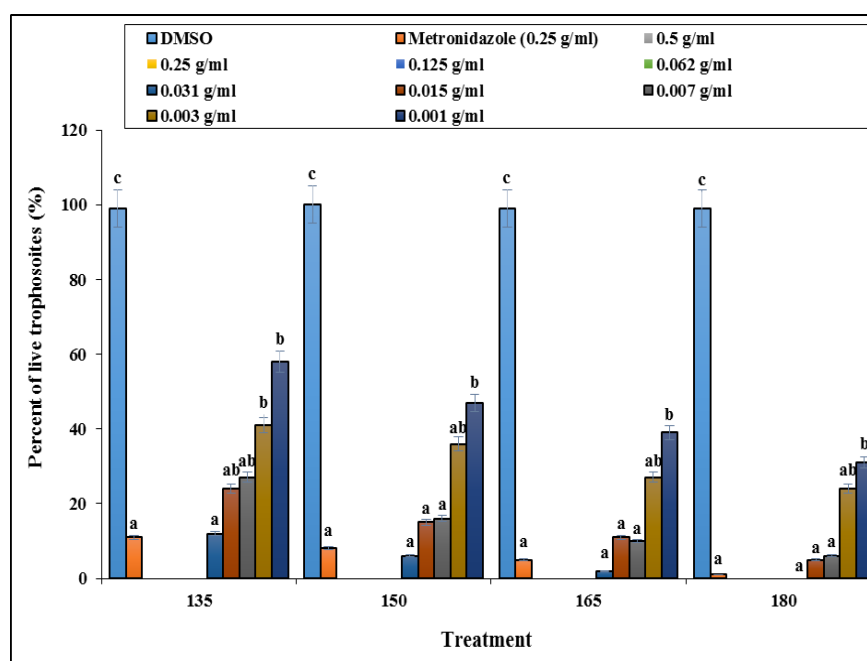


Figure 3. Effect of essential oil of *C. sativum* on the percent of live *T. vaginalis* trophozoites in 135, 150, 165 and 180 minutes. Non-identical letters indicate a significant difference between the groups ($p \leq 0.05$).

0.015, 0.007, 0.003, 0.001 g/ml concentrations of essential oil and metronidazole showed 12, 24, 27, 41, 58, and 11% of percent of live trophozoite after 135 minutes (Fig. 3). The 0.031, 0.015, 0.007, 0.003 and 0.001 g/ml concentrations of the *C. sativum* essential oil showed 100 % death of the trophozoite after 180, 240, 300, 360 and 420 minutes of incubation, respectively (Fig. 3,4). While metronidazole at the 0.25 g/ml concentration destroyed the trophozoite completely after 240 minutes of incubation. No anti-parasitic effect

was observed due to DMSO during the experiment.

MIC and MLC determination of *C. sativum* essential oil

In the examined parasite, *C. sativum* essential oil with 0.015 g/ml concentration inhibited *T. vaginalis* trophozoite growth (MIC) and with 0.031 g/ml concentration destroyed it (MLC) (Table 2).

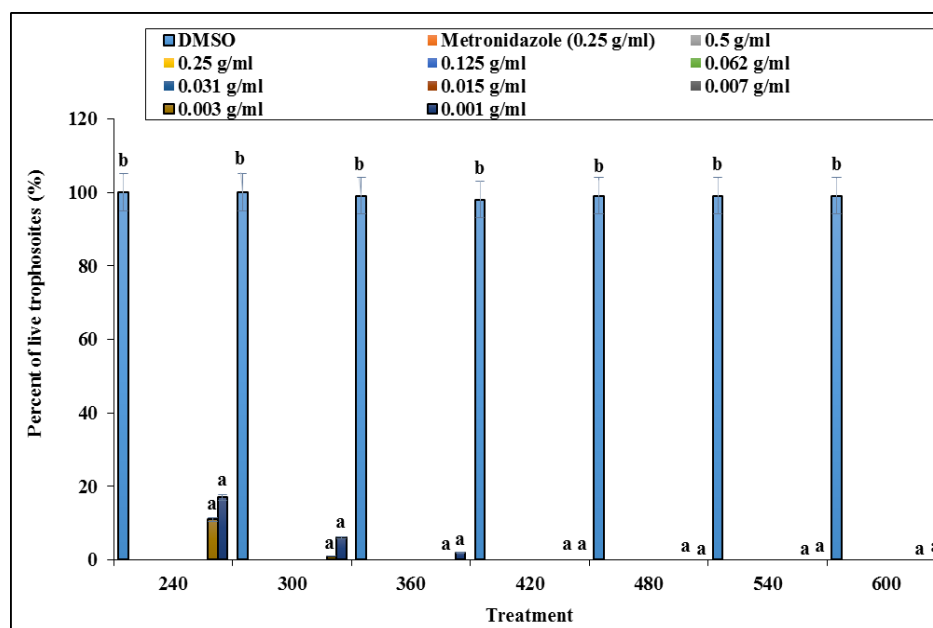


Figure 4. Effect of essential oil of *C. sativum* on the percent of live *T. vaginalis* trophozoites in 240, 300, 360, 420, 480, 540 and 600 minutes.

Non-identical letters indicate a significant difference between the groups ($p \leq 0.05$).

Table 2. MIC and MLC of essential oils of *C. sativum* ($p < 0.01$)

Microorganism	<i>T. vaginalis</i> trophozoite
MIC (g/ml)	0.015
MLC (g/ml)	0.031

DISCUSSION

Volvo-vaginitis as a usual medical problem can lead to remarkable inconvenience and repeated medical visits. Infection, allergy, and systemic diseases can happen due to Volvo-vaginitis. Its main causes before menopause are bacterial vaginitis, Volvo-vaginal candidiasis, and *T. vaginalis* [24]. *T. vaginalis* causes vaginitis in women and rarely urethritis and sometimes prostatitis in men. The symptoms either do not or rarely appear in men and starts with discharge and itching in women. Given the importance of the disease and the problems of controlling and combating due to sexual transmission, the drug resistance and the lack of appropriate and certain vaccines, a need is felt for appropriate, safe, inexpensive and accessible medical approaches [25]. One way to prevent and control the resistance of pathogenic microorganisms to the antibiotic is using ethno medicinal plants. *C. sativum* as a plant in Iranian traditional medicine has been indicated to have some optimal treatment properties, due to its antioxidant and anti-inflammatory effects in both in vitro and in vivo [21,22]. But, to our knowledge, this is the first time essential oil of *C. sativum* collected in Kermanshah has been used for its anti-trichomoniasis effects.

Yield and analysis of essential oil of *C. sativum*

The essential oil yield of the *C. sativum* essential oil was 0.63%, calculated on the fresh plant. Analysis of the obtained essential oil from the leaf of *C. sativum* by GC-MS

leads to the identification of fifteen components, representing 99.2% of the total essential oil (Table 1). Regarding the chemical constituents, their relative percentage of the total chromatogram area and retention index as it had been shown in table 1. α -pinene, β -pinene, p-cymene, limonene, γ -terpinene, linalool, terpinen-4-ole, decanal, nerol, carvacrol, thymol, neryl acetate, 2e-dodecanal, tetrahydro ionol, n-hexadecanewere identified in the essential oil of *C. sativum*. The main component of *C. sativum* was linalool (71.2%). Linalool as the main component in *C. sativum* refers to two enantiomers of a naturally occurring terpene alcohol found in several aromatic plants such as *Lavandula*, *Cinnamomum tamala*, *Cannabis sativa*, *Cannabis indica*, *Ocimum basilicum*, *Solidago Meyen*, *Solidago chilensis*, *Artemisia vulgaris*, and *Humulus lupulus* [26-29]. The composition of the essential oil of *C. sativum* in some of the world has been studied and found differ from each other. In a study indicated that essential oil of *C. sativum* contained 44 compounds mostly of aromatic acids containing capric acid, 2-decenoic acid, undecyl alcohol, undecanoic acid, tridecanoic acid and E-11-tetradecenoic acid and as major constituents [30]. In other study demonstrated essential oil of *C. sativum* is rich of methyl heptenol, elemol, caryophyllene oxide, geranyl acetate, linalyl acetate, thymol, geraniol, citronellol, β -caryophyllene, borneol, eucalyptol, β -phellandrene, limonene, and α -pinene [31]. In agreement with our results, it is reported that the essential oil of *C. sativum* contains linalool

(60-70%) [32]. Also in other studies revealed linalool as major constituents of essential oil of *C. sativum* [33-35].

Anti-trichomoniasis property of essential oil of *C. sativum*

In this study, the anti-parasitic properties of *C. sativum* essential oil on *T. vaginalis* trophozoite were assessed. The result indicated that after 420 minutes of incubation of the parasite with several concentrations of essential oil had 100% death of trophozoite. According to the results, the essential oil demonstrated the highest anti-parasitic effect on trophozoite at 0.5 and 0.25 g/ml concentrations. Another main characteristic of our study was that *C. sativum* essential oil with 0.015 g/ml concentration inhibited *T. vaginalis* trophozoite growth and with 0.031 g/ml concentration destroyed it. There are several studies about the antiparasitic effect of *C. sativum*, but there isn't study about the anti-trichomoniasis effect of it [36-38]. In studies indicated *C. sativum* have antiparasitic effects against *Tribolium confusum* and *Callosobruchus maculatus* [36, 37]. Also in other study demonstrated the anthelmintic property of *C. sativum* against *Pheretima posthumad* [38]. Indeed, the anti-parasitic effect of the *C. sativum* is related to its constituent constituents. In our study, the main compound in the essential oil was linalool. In studies indicated linalool have good antibacterial and antiparasitic effect and can use as the antimicrobial supplement or drug [39-41].

CONCLUSION

In this study, essential oil of *C. sativum* as an aromatic medicinal plant possess anti-trichomonas effect against *T. vaginalis* trophozoite. The trophozoite was destroyed completely by the several doses of examined essential oil (The best results were observed at 0.5 and 0.25 g/ml concentrations of essential oil). Also, the results indicate that essential oil of *C. sativum* has its chemical composition, which is attributed to its anti-trichomoniasis activity. These compounds (especially linalool) can be used as anti-trichomoniasis supplement or drug. Fractionation and characterization of active molecules will be the future work to investigate.

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CONFLICTS OF INTEREST

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article

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