Chemical composition and insecticidal activity of *Cymbopogon citratus* essential oil from Cuba and Brazil against housefly

Composição química e atividade inseticida do óleo essencial de *Cymbopogon citratus* de Brasil e Cuba contra mosca doméstica

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Abstract

Essential oil of *Cymbopogon citratus* collected from Brazil and Cuba was tested to a chemical characterization and then was tested on the post-embryonic development of *Musca domestica*. The chemical composition analysis by GC-MS of the oils from Brazil/Cuba allowed the identification of 13 and 12 major constituents respectively; nine of them common to both. In the both oils, the main components were the isomers geranial and neral, which together form the compound citral. This corresponds to a total of 97.92%/Brazil and 97.69%/Cuba of the compounds identified. The monoterpene myrcene, observed only in the sample of Cuba, presented a large relative abundance (6.52%). The essential oil of *C. citratus* (Brazil/Cuba) was dissolved in DMSO and tested at concentrations of 5, 10, 25, 50, 75 and 100% and citral was prepared by mixing 16.8 mg with 960 µL DMSO. Both essential oils and monoterpene citral were applied topically to newly-hatched larvae (1µL/larva). The results showed a lethal concentration (LC50) of 4.25 and 3.24% for the Brazilian and Cuban essential oils, respectively. Mortalities of larval and newly-hatched larvae to adult periods were dose-dependent for the two both oils as for monoterpene citral, reaching 90%. Both essential oils and citral caused morphological changes in adult specimens.

Keywords: Vector control, essential oil, house-fly, lemongrass, *Cymbopogon citratus*.

Resumo

O óleo essencial de *Cymbopogon citratus*, coletado no Brasil e em Cuba, foi caracterizado quimicamente e testado no desenvolvimento pós-embrionário de *Musca domestica*. A análise da composição química dos óleos essenciais (Brasil/Cuba), por Cromatografia Gasosa acoplada ao espectrômetro de massa (GC-EM), permitiu a identificação de 13 e 12 componentes principais, respectivamente; nove deles comuns aos dois. Em ambos os óleos, os principais componentes foram os isômeros geranial e neral, que, juntos, formam o composto citral. Esse corresponde a um total de 97,92%/Brasil e 97,69%/Cuba dos compostos identificados. O monoterpeno mirceno, observado na amostra cubana, apresentou grande abundância relativa (6,52%). O óleo de *C. citratus* (Brasil/Cuba) foi dissolvido em DMSO, obtendo-se as concentrações de 5, 10, 25, 75 e 100%; e o citral (16,8 mg) foi misturando com 960mL de DMSO. Tanto o óleo essencial como o monoterpeno citral foram aplicados topicalmente nas neolarvas (1µL/larva). Os resultados mostraram uma concentração letal (CL50)
de 4,25% e 3,24% para o óleo essencial brasileiro e cubano, respectivamente. As mortalidades do período larval e o de neo-larva a adulto foram dose-dependentes, tanto para os óleos como para o monoterpeno citral, podendo chegar a 90%. Ambos os óleos essenciais e citral causaram alterações morfológicas nos espécimes adultos.

**Palavras-chave:** Controle de vetores, óleo essencial, mosca doméstica, lemongrass, Cymbopogon citratus.

### Introduction

The use of chemical insecticides in pest control induces insect resistance, and impact the environment through water and soil contamination, becoming toxic to vertebrates (PRADO, 2003). Thereby, multiple worldwide efforts to use botanical products to control insect vectors and pests appeared in the latest years. Biopesticides offer an alternative to insect control in which the damage to the environment is minimized, reaching only target organisms, with a minimal residual activity against predators, parasites and pollinator insects (LIU et al., 2000), making its use appropriate in integrated pest management programs (ISMAN, 2006; KOUL et al., 2008).

Plants and their natural enemies (insects, bacteria or viruses) have undergone a co-evolution process in which a new plant resistance character that reduces enemy attack is developed. The essential oils are a type of metabolite with this function, characterized by complex mixtures of monoterpenoids and sesquiterpenoids as major metabolites. The number and quantities of compounds in the essential oil produced by a single plant can change with the environment characteristic, place of collection, plant age and other conditions, but in general, the major compounds remain as a significative chemical marker. Due to the volatile, odorous and lipophylic characteristics of the essential oils, they can be toxic to insects, induce behavioral modifications, provoke direct disruption of specific physiological routes related to neuroendocrine system and in their reproduction (PRATES & SANTOS, 2002; GARCIA & AZAMBUJA, 2004). In addition, essential oils have been shown to be relatively non-toxic to fish, birds and mammals and easily biodegrade in the environment (KUMAR et al., 2012), turning them into good biopesticides.

Diptera Muscoid presents a great medical-sanitary importance and is closely related to animals and human environment, acting as an important vector of pathogens, such as bacteria, protozoa cysts and oocysts, helminthes, fungi and viruses (VAZIRIANZADEH et al., 2008; BARIN et al., 2010), besides being responsible for the production of myiasis in humans and animals (ZUMPT, 1985). The immature stages of some species of these flies develop in animal and plant decaying organic matter such as feces, garbage, corpses and carrion (GRABOVAC & PETRIĆ, 2003).

Some studies revealed satisfactory results from the use of several essential oils for insect management such as the cosmopolitan pest house fly, Musca domestica L. (PAVELA, 2008); malarian vector mosquito, Anopheles gambiae Giles (McALLISTER & ADAMS, 2010); parasitic mites of the honeybees bee Varroa destructor (ANDERSON & TRUEMAN, 2000); Acari: Varroidae (GHASEMI et al., 2011); and the maize weevil adults, Sitophilus zeamais Motschulky (FAZOLIN et al., 2007).

The essential oil of Cymbopogon citratus (DC) Stapf (Poaceae), most known as “lemongrass”, is commonly used by folk medicine in many countries. Native from India and Southeast Asia, it is distributed in numerous tropical countries, including Brazil (DUARTE & ZANETI, 2004; SOUSA et al., 2010). There are several popular uses for this plant, including treatment for stomach pains, diarrhea (TANGPU & YADAV, 2006), also having several pharmacological activities such as anti-amoebic and as antifungal (SHAH et al., 2011). Also it has been reported as potentially useful against insects (CAVALCANTI et al., 2004; KUMAR et al., 2013). Recently, some studies revealed that C. citratus essential oil and its main components (citral and 1.8 cineole), are important repellent and insecticide against housefly, but these studies are focused mainly in the instant effectiveness after application and not in long time effect. (KUMAR et al., 2011b, 2013; SINTHUSIRI & SOONWERA, 2013). As of today, no study considered the effect of the essential oil in all the stages of the fly’s life cycle; that’s why it became important to reveal the effect of those essential oils in the post-embryonic development of M. domestica.

This report describes the evaluation of the chemical composition and insecticidal activity of C. citratus essential oil collected in Brazil and Cuba and its major compound (Citral) on the post-embryonic development of M. domestica.

### Materials and Methods

#### Plant collection and identification

The Brazilian lemongrass fresh leaves were collected at Laboratory of Cultivation and Biomass Production of Farmaguinhos/Fiocruz- Jacarepaguá campus, Rio de Janeiro, Brazil (22°87’49”S, 43°24’53”W). A voucher specimen was deposited at Rio de Janeiro Botanical Garden Herbarium (RB) under the number RB3273021. The Cuban specimen was collect in the district of Mirafl ores, municipality of Moa, Holguín, Cuba (20°38’21”N-75°01’44”W). The identification of the species and the quality parameters of vegetable drugs were secured by the Company of Agriculture municipality of Moa, the exclusive plant provider in this region of Cuba. A voucher specimen was deposited at BSC Herbarium under the number 16443.

#### Extraction and component characterization by Gas Chromatographic mass spectrometry (GC-MS) analysis

Fresh leaves of C. citratus were extracted by hydrodistillation using a “Clevenger type apparatus”, as recommended by the Anvisa (2010). The chemical composition analysis of C. citratus oils (Brazil and Cuba) was done by High Resolution Gas Chromatography (HRGC) coupled to a mass spectrometer (MS). The gas chromatograph equipment model HP7590 (Agilent
Technologies, USA), equipped with DB-5MS capillary column produced by the same company with dimensions 30 m × 0.32 m and 0.25 mm thick film, was used. The program temperature conditions consisted in a temperature program from 40 °C until 290 °C, with an increment of 4 °C/min. The injection volume of the sample was 1 µL with a split ratio of 100:1, using helium as the carrier gas at a flow rate of 0.5 ml per minute. Both injector and detector temperature were maintained at 290 °C. The percentage composition was calculated using peak normalization method assuming equal detector response. The samples were then analyzed by a quadrupole mass spectrometer model HP5972 A with an electron impact ionization at 70 eV. The compounds separated were characterized from their mass spectral data using the National Institute of Standards and Technology mass spectrometry library (ADAMS, 2007) and according with their Kovat retention indexes.

House-fly colony

Specimens were collected on the campus of Fundação Oswaldo Cruz, Rio de Janeiro, and were reared and maintained in the Laboratório de Transmissores de Leishmanioses - Setor de Entomologia Médica e Forense of the same Institution following the methodology used in previous works according to Queiroz & Milward-de-Azevedo (1991). Flies were kept in cages at room temperature with water and sugar ad libitum. Decaying bovine ground beef was given for the maturation of the ovarioles and to stimulate oviposition. The second generation was reared following the same methodology and newly hatched larvae were used in the experiments.

Bioassay

Serial dilutions were performed from essential oils of C. citratus from Brazil and Cuba dissolved in dimethyl sulfoxide (DMSO) (SIGMA - USA) to obtain six different test concentrations: 5% (28 x 280)(25 µL/oil + 475 µL/DMSO); 10% (50 µL/oil + 450 µL/DMSO); 25% (125 µL/oil + 375 µL/DMSO); 50% (250 µL/oil + 250 µL/DMSO); 75% (375 µL/oil + 125 µL/DMSO) and 100% (pure oil). Citral (purchased from Tedia® - Brasil) was prepared by mixing 16.8 mg with 960 µL DMSO.

Both essential oils (Brazil’s and Cuba’s) and citral were applied topically (1 µL/larva) to newly hatched larva bodies of M. domestica using micropipettes. In all experimental groups each concentration was performed in quadruplicate using fifty newly-hatched larvae per experimental group. In addition, two control groups were performed (with/without DMSO). After treatment, the newly-hatched larvae were transferred and placed onto recipients with 50g of putrefied bovine meat (1g/larva), to guarantee enough food for maximum development. These recipients were placed into larger ones (500 mL) containing a substrate for pupation and food for maximum development. These recipients were placed into climated chambers set at 27±1°C, 70±10% RH, 12:12 light/dark cycle. Daily observations were made until the emergence of the adults, with subsequent sex ratio calculation (nFemale/nMale) (RODRIGUES, 2004) and morphologic deformities analysis. Viability and duration of each period (larval, pupal and newly-hatched larvae to adult) were analyzed. Another variable considered was the weight of mature larvae. Results were analyzed by One-way Analysis of Variance (ANOVA) (P<0.0001), and mean values were compared by the Tukey-Kramer test at significance level of 0.05 (ZAR, 1999). Values of LC50 and LC90 were computed with Microsoft Office Excel Program.

Results and Discussion

Chemical characterization of essential oil

Compounds identified in C. citratus essential oils from Brazil and Cuba are presented in Table 1. GC/MS analysis allowed the identification of 13 and 12 main chemical components for Brazilian and Cuban oils, respectively. In both of them, the major components were the isomers geranial with 53.2 and 51.14% and neral with 36.37 and 35.21% for Brazilian and Cuban samples, respectively. Besides that, other 8 compounds appear in common. The monoterpene myrcene (6.52%), observed in Cuban sample, was the only differentiating compound that is present in high relative abundance.

Chemical studies of C. citratus in different habitats around the world identified citral as the major volatile constituent (SOLÓRZANO-SANTOS & MIRANDA-NOVALES, 2012).

Table 1. Chemical composition (%) of essential oils from fresh leaves of Cymbopogon citratus (DC) Stafn natives from Brazil and Cuba.

| Constituents                  | Percentage composition | Kovat’s ID | Brazil | Cuba |
|-------------------------------|------------------------|------------|--------|------|
| 6-methylhept-5-en-2-one       | 0.19                   | 936        | MS, RI |      |
| Camphene                      | 0.29                   | 953        | MS, RI |      |
| Merycne                       | -                      | 6.52       | 988    | MS, RI|
| Limonene                      | 0.99                   | 1030       | MS, RI |      |
| Linalool                      | 0.42                   | 1079       | MS, RI |      |
| Citronellal                   | -                      | 0.16       | 1132   | MS, RI|
| n-decanol                     | 0.19                   | 1214       | MS, RI |      |
| Z-citrat (Neral)              | 36.37                  | 1231       | MS, RI |      |
| Geraniol                      | 2.66                   | 1247       | MS, RI |      |
| E-citrat (geranial)           | 53.2                   | 1258       | MS, RI |      |
| 2-undecanone                  | 0.22                   | 0.35       | 1287   | MS, RI|
| geranyl acetate               | 1.5                    | 0.20       | 1359   | MS, RI|
| (E)-caryophyllene             | 1.03                   | -          | 1414   | MS, RI|
| 2-tridecanone                 | -                      | 0.10       | 1486   | MS, RI|
| γ-cadinene                    | 0.27                   | 0.27       | 1513   | MS, RI|
| Caryophyllene oxide           | 0.59                   | 0.59       | 1583   | MS, RI|
| Total identified              | 97.92                  | 97.69      |        |      |

ID = Identification methods; MS = comparison of the mass spectrum with those of the computer mass libraries, and Adams (2007); Kovat’s R.I. = Kovat’s Retention Index with those reported in the literature.
Table 2. Duration (days) of post-embryonic development of *Musca domestica* from Brazil and Cuba and monoterpane citral, under laboratory conditions.

| Treatments | Larval stage | Pupal stage | Newly-hatched larvae to adult |
|------------|--------------|-------------|------------------------------|
| Control    | Brazil: 6.68±0.47\(^a\) | Cuba: 6.68±0.47\(^a\) | Brazil: 5.23±0.42\(^a\) | Cuba: 5.23±0.42\(^a\) | Brazil: 11.91±0.29\(^a\) | Cuba: 11.91±0.29\(^a\) |
| DMSO       | Brazil: 5.31±0.58\(^b\) | Cuba: 5.31±0.58\(^b\) | Brazil: 5.31±0.58\(^b\) | Cuba: 5.31±0.58\(^b\) | Brazil: 10.63±1.16\(^c\) | Cuba: 10.63±1.16\(^c\) |
| 5%         | Brazil: 7.17±0.37\(^d\) | Cuba: 7.14±0.35\(^d\) | Brazil: 7.14±0.34\(^d\) | Cuba: 7.14±0.34\(^d\) | Brazil: 14.51±0.50\(^d\) | Cuba: 14.42±0.49\(^d\) |
| 10%        | Brazil: 7.20±0.40\(^e\) | Cuba: 7.15±0.36\(^e\) | Brazil: 7.11±0.31\(^e\) | Cuba: 7.10±0.30\(^e\) | Brazil: 14.49±0.50\(^e\) | Cuba: 14.39±0.49\(^e\) |
| 25%        | Brazil: 7.18±0.39\(^f\) | Cuba: 7.17±0.38\(^f\) | Brazil: 7.15±0.36\(^f\) | Cuba: 7.13±0.34\(^f\) | Brazil: 14.44±0.50\(^f\) | Cuba: 14.43±0.49\(^f\) |
| 50%        | Brazil: 5.18±0.39\(^g\) | Cuba: 5.19±0.39\(^g\) | Brazil: 7.14±0.34\(^g\) | Cuba: 7.10±0.31\(^g\) | Brazil: 12.55±0.50\(^g\) | Cuba: 12.46±0.50\(^g\) |
| 75%        | Brazil: 5.20±0.40\(^h\) | Cuba: 5.23±0.42\(^h\) | Brazil: 7.17±0.37\(^h\) | Cuba: 7.23±0.42\(^h\) | Brazil: 12.56±0.50\(^h\) | Cuba: 12.50±0.50\(^h\) |
| 100%       | Brazil: 12.15±0.36\(^i\) | Cuba: 12.18±0.39\(^i\) | Brazil: 7.20±0.40\(^i\) | Cuba: 7.17±0.38\(^i\) | Brazil: 19.52±0.51\(^i\) | Cuba: 19.50±0.50\(^i\) |
| Control    | Brazil: 6.50±0.51\(^a\) | Cuba: 6.50±0.51\(^a\) | Brazil: 5.37±0.49\(^a\) | Cuba: 5.37±0.49\(^a\) | Brazil: 11.85±0.36\(^a\) | Cuba: 11.85±0.36\(^a\) |
| DMSO       | Brazil: 5.29±0.47\(^b\) | Cuba: 5.29±0.47\(^b\) | Brazil: 5.27±0.45\(^b\) | Cuba: 5.27±0.45\(^b\) | Brazil: 10.54±0.90\(^b\) | Cuba: 10.54±0.90\(^b\) |
| Citral     | Brazil: 3.18±0.40\(^c\) | Cuba: 3.18±0.40\(^c\) | Brazil: 4.91±0.30\(^c\) | Cuba: 4.91±0.30\(^c\) | Brazil: 7.91±0.30\(^c\) | Cuba: 7.91±0.30\(^c\) |

*Values within a column followed by the same letter are not significantly different at the 5% level according to Tukey’s HSD. Oil test with four replication, N=50 and Citral test with three replication, N=10. DMSO= dimethylsulfoxide.*
essential oils and its constituents is unknown, the appearance of toxic signs is fast (KNAAK & FIUZA, 2010).

Any of these observations could explain the differences in development time and mortality of *M. domestica* treated with pure citral and citral found in the essential oil diluted in DMSO.

Sex ratio did not differ significantly in any of the treated groups when compared to control groups (Table 3). Larval weight from Brazil and Cuba showed significant difference (p < 0.0001) when compared to control groups.

Lightest larvae (17.53mg oil/Brazil and 17.49mg oil/Cuba) belong to concentration of 10% while the heaviest larvae (27.01mg oil/Brasil and 26.97mg oil/Cuba) belong to the concentration of 50%, when compared to control groups with DMSO (21.59mg) and without DMSO (21.50mg). Monoterpene citral significantly increased larval weight (25.65mg) when compared to control groups with DMSO (22.22mg) and without DMSO (21.18mg) (Table 3).

Necrophagous Diptera are more suitable to pupate even when the final weight is below the average estimated for other species (MENDONÇA et al., 2011). According to Lomonaco & Germanos (2001), the increasing in the development period may be due to delays in obtaining the necessary weight for pupating (ROPER et al., 1996), due to the difficulties in obtaining food. These data are similar to some of the results obtained in this experiment.

Mortality of *M. domestica* in larval, pupal and newly-hatched larvae to adult periods was affected in a dose-dependent manner for both oils. Mortality of newly-hatched larvae showed highly significant values for Brazil and Cuba, respectively: 5% (62.5 / 61.5); 10% (62.0 / 63.0); 25% (64.0 / 65.0); 50% (75.0 / 73.0); 75% (77.5 / 78.0) and 100% (87.5 / 87.0) (Figure 1, 2).

Monoterpene citral presented a slightly higher mortality at all development stages when compared to essential oil of *C. citratus*

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**Table 3.** Larval weight (mg) and sex ratio of *Musca domestica* (Diptera:Musciidae) treated with essential oil of *Cymbopogon citratus* from Brazil and Cuba and monoterpene citral, under laboratory conditions.

| Treatments     | Brazil (Mean ± SD)* | Brazil (Mean ± SD) | Cuba (Mean ± SD) | Cuba (Mean ± SD) | Sex ratio |
|----------------|---------------------|---------------------|-------------------|-------------------|-----------|
| Control        | 21.50±1.76º         | 21.50±1.76º         | 19.00 – 24.40      | 19.00 – 24.40      | 0.50      |
| DMSO           | 21.59±1.39º         | 21.59±1.39º         | 15.00 – 24.20      | 15.00 – 24.20      | 0.51      |
| 5%             | 19.60±1.41b         | 19.50±1.32b         | 17.20 – 21.50      | 17.50 – 21.00      | 0.50      |
| 10%            | 17.53±1.23c         | 17.49±1.27c         | 15.80 – 20.60      | 15.60 – 20.60      | 0.51      |
| 25%            | 20.37±0.95d         | 20.36±0.93d         | 19.40 – 21.80      | 19.40 – 21.80      | 0.51      |
| 50%            | 27.01±2.18e         | 26.97±2.38e         | 21.90 – 33.00      | 24.80 – 33.00      | 0.54      |
| 75%            | 21.50±2.65f         | 21.35±2.69f         | 17.40 – 25.30      | 17.20 – 25.20      | 0.53      |
| 100%           | 22.05±1.65g         | 22.39±2.19g         | 19.50 – 25.60      | 19.00 – 25.60      | 0.53      |

*Values within a column followed by the same letter are not significantly different at the 5% level according to Tukey’s HSD. DMSO= dimethylsulfoxide. Oil test with four replication, N=50 and citral test with three replication, N=10.

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**Figure 1.** Mortality of larval, pupal and newly-hatched larvae to adult periods of *Musca domestica* after exposure to different concentrations of *Cymbopogon citratus* oil from Brazil, under laboratory conditions.
Insecticidal activity of Cymbopogon citratus oil (Figures 1, 2 and 3). This can be explained by the fact that insecticidal activity of this essential oil has been attributed to its major monoterpenic citral (YANG et al., 2005).

Kumar et al. (2011b) testing several essential oils against M. domestica, also noted a high rate of mortality after 48h exposure. For C. citratus the authors found a mortality rate of 77%. An contact toxicity bioassay of C. citratus against M. domestica larvae and pupae showed lethal concentration (LC$_{50}$) value of 0.41 µl/cm$^2$ and a percentage inhibition rate (PIR) of 7% and 7.3%, respectively (KUMAR et al., 2013). Abdel Halim & Morsy (2006) also observed high mortalities in Muscidae after using essential oils of Cupressus macrocarpa Hartw. (Cupressaceae) and Alpinia officinarum Hance (Zingiberaceae) against Synthesiomyia nudiseta (Wulp, 1883) (Muscidae: Azeliinae).

C. citratus essential oil showed a LC$_{50}$ of 4.25 and 3.24% and a LC$_{90}$ of 84.25 and 83.24% for Brazil and Cuba, respectively. Different concentrations of citral presented a significant larval mortality, with LC$_{50}$ of 0.19 and 0.09 µl/cm$^3$ after 24 and 48h, respectively, and the other monoterpen 1,8-Cineole LC$_{50}$ of 0.36 and 0.15 µl/cm$^3$ for the same interval time (KUMAR et al., 2013). Khater et al. (2011) working with Egyptian essential oils showed a high effectiveness against Lucilia sericata (Meigen, 1826) (Diptera: Calliphoridae) with LC$_{50}$ values of 0.57, 0.85, 2.74 and 6.77% for lettuce, chamomile, anise and rosemary, respectively, slowing larval growth at sublethal concentrations. Dipping assay using lemongrass demonstrated LC$_{50}$ of 69ppm against Aedes aegypti (Linnaeus, 1972) (Diptera:Culicidae) and C. quinquefasciatus larvae a LC$_{50}$ of 144ppm. The essential oil of C. citratus showed to have a great larvicidal activity against A. aegypti and caused 100%

Figure 2. Mortality of larval, pupal and newly-hatched larvae to adult periods of Musca domestica after exposure to different concentrations of Cymbopogon citratus oil from Cuba, under laboratory conditions.

Figure 3. Mortality of larval, pupal and newly-hatched larvae to adult periods of Musca domestica after exposure to Citral, under laboratory conditions.
Table 4. Percentage (%) of morphological deformities from adults of *Musca domestica* treated with essential oil of *Cymbopogon citratus* from Brazil and Cuba and monoterpane citral, under laboratory conditions.

| Treatments | Morphological deformities (%) |
|------------|-------------------------------|
| Brazil     | Cuba                         |
| Control    | 0.00                          | 0.00 |
| DMSO       | 0.00                          | 0.00 |
| 5%         | 38.67                         | 37.66 |
| 10%        | 55.26                         | 56.76 |
| 25%        | 76.39                         | 78.57 |
| 50%        | 81.13                         | 80.65 |
| 75%        | 86.67                         | 88.64 |
| 100%       | 100.00                        | 100.00 |
| Control    | 0.00                          | 0.00 |
| DMSO       | 0.00                          | 0.00 |
| Citral     | 100.00                        | 100.00 |

DMSO= dimetilsulfoxide. Oil test with four replication, N=50 and citral test with three replication, N=10.

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