Research Article

Essential oils activity from plants of the Brazilian Caatinga on the vegetable leafminer[1]

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ABSTRACT

Liriomyza sativae (Blanchard) is a worldwide polyphagous pest for horticultural and ornamental crops, whose chemical control is the major method adopted. However, botanical insecticides in the form of essential oils (EOs) are presented as promising alternatives. This study aimed to evaluate the effect of EOs from the species *Croton sonderianus* Muell. Arg., *Croton conduplicatus* Kunth., *Lippia gracilis* Schauer and *Lippia schaueriana* Mart. on the biological aspects of *L. sativae* adults and immature stages (larva and pupa). The results showed larval and pupal mortality after the immersion of leaves with newly hatched *L. sativae* larvae in EOs solution from *L. gracilis* and *L. schaueriana* leaves. The EOs from *C. conduplicatus* stem bark and leaf and from *C. sonderianus* stem bark extended the leafminer pupal stage duration, while those from *C. conduplicatus* leaves and *C. sonderianus* stem bark decreased the oviposition and feeding punctures in no-choice tests. Only *C. conduplicatus* confirmed the effect in the free-choice test, showing to be the most promising in the study. This way, EOs from *L. gracilis* and *L. schaueriana* leaves show an insecticide activity on *L. sativae* larvae, and those from *C. conduplicatus* leaves reduce the leafminer oviposition and feeding punctures in melon plants.

KEYWORDS: *Liriomyza sativae*, *Croton*, *Lippia*, botanical insecticides, melon.

RESUMO

Atividade de óleos essenciais de plantas da Caatinga sobre a mosca-minadora

Liriomyza sativae (Blanchard) é uma praga polífaga de distribuição mundial em hortaliças e plantas ornamentais, cujo controle químico é o principal método adotado. Entretanto, inseticidas botânicos na forma de óleos essenciais (OEs) são apresentados como alternativas promissoras. Objetivou-se avaliar o efeito de OEs das espécies *Croton sonderianus* Muell. Arg., *Croton conduplicatus* Kunth., *Lippia gracilis* Schauer e *Lippia schaueriana* Mart. sobre aspectos biológicos de adultos e estágios imaturos (larva e pupa) de *L. sativae*. Os resultados demonstram mortalidade larval e pupal após imersão de folhas com larvas recém-eclodidas de *L. sativae* em soluções de OEs de folhas de *L. gracilis* e *L. schaueriana*. Os OEs de casca e folha de *C. conduplicatus* e de casca de *C. sonderianus* prolongaram o período de pupa da mosca-minadora, enquanto os de folhas de *C. conduplicatus* e casca de *C. sonderianus* causaram redução na oviposição e puncturas de alimentação em testes de confinamento. Apenas *C. conduplicatus* confirmou o efeito no teste com livre chance de escolha, mostrando-se como o mais promissor do estudo. Desta forma, OEs de folhas de *L. gracilis* e *L. schaueriana* possuem atividade inseticida sobre larvas de *L. sativae* e os de folhas de *C. conduplicatus* reduzem a oviposição e as puncturas de alimentação de mosca-minadora em meloeiro.

PALAVRAS-CHAVE: *Liriomyza sativae*, *Croton*, *Lippia*, inseticidas botânicos, melão.

INTRODUCTION

The leafminer *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) is a polyphagous pest of vegetables and ornamental plants, originally from the Americas; however, it is currently a global pest present in Africa, Europe, Asia and Oceania (CABI 2018).

The major damage is caused by its larvae, which feed internally in the leaves, forming galleries. Thus, there is a leaf area damage and, consequently, a photosynthetic activity reduction, resulting in a lower yield and fruit quality (Costa et al. 2017).

Chemical control is the major method adopted by producers; however, insecticide-resistant populations have been reported (Ferguson 2004, Wei 2015). Also, parasitoids are the major natural enemies of leafminers (Connor & Taverner 1997), and several studies have shown the insecticides impact on these species (Matsuda & Saito 2014, Guantai et al. 2015).

1. Received: Apr. 25, 2019. Accepted: July 19, 2019. Published: Dec. 11, 2019. DOI: 10.1590/1983-40632020v5058313.
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As an alternative for the synthetic chemical compounds, strategies are being studied in melon crops to control *L. sativae*, such as biological control with parasitoids (Costa-Lima et al. 2019), plant resistance (Celin et al. 2018) and botanical insecticides (Costa et al. 2018). The latter is based on plant materials, extracts or natural products derived from plants (Isman et al. 2011). Due to presenting a complex of substances that allow a greater interaction and acting on multiple targets, these products present a lower risk of selecting populations resistant to its compounds (Ntalli & Menkissoglu-Spiroudi 2011). Among the botanical insecticides, Isman et al. (2011) suggest that plant essential oils (EOs) should become a popular choice for pest management. Among the positive aspects, the authors highlight that the EOs are generally non-toxic to mammals and environmentally non-persistent.

Biomes with a high biodiversity and low scientific exploration may be a good source for discovering new species that can act as botanical insecticides. The Caatinga, in northeastern Brazil, is the largest and most diverse neotropical seasonally dry tropical forest (Werneck et al. 2011). This biome has registered 4,657 seed plant species, with 913 (19.7 %) identified as endemic (Zappi et al. 2015). Among these endemic Caatinga plants, EOs from the *Lippia* and *Croton* genus have been studied for their chemical composition (Almeida et al. 2014, Souza et al. 2017a, Souza et al. 2017b, Souza et al. 2018). These studies showed the presence of compounds in these EOs that have been reported in the literature as presenting an insecticidal activity or effect on the insect behavior, such as carvacrol (Bagul et al. 2018), piperitone oxid (Grudniewska et al. 2011), eucalyptol (Moretti et al. 2015) and α-pinene (Haselton et al. 2015).

Thus, this study aimed to evaluate the effect of EOs from the species *Croton sonderianus* Muell. Arg. (leaves and stem bark), *Croton conduplicatus* Kunth. (leaves and stem bark), *Lippia gracilis* Schauer (leaves) and *Lippia schaueriana* Mart. (leaves) on *L. sativae* adults and immature stages (larvae and pupae) biological aspects.

MATERIAL AND METHODS

L. gracilis and *L. schaueriana* leaves and *C. sonderianus* and *C. conduplicatus* leaves and stem barks were collected in Petrolina (Pernambuco state, Brazil), respectively in the coordinates 09º23'35"S and 40º30'27"W; 09º09'S and 40º22'W; 09º07'17"S and 40º31'9"W; 09º03'54"S and 40º19'12"W. The *Lippia* spp. vegetative structures were collected in December 2011 and the *Croton* spp. samples between July and September 2012. The essential oils were obtained according to Souza et al. (2017a).

The identification of compounds present in the EOs samples used in this study was previously published for the following species: *C. sonderianus* leaves (Souza et al. 2017a), *C. conduplicatus* leaves (Almeida et al. 2014) and stem barks (Oliveira et al. 2017), *L. gracilis* leaves (Souza et al. 2017b) and *L. schaueriana* leaves (Souza et al. 2018). For the *C. sonderianus* stem barks EOs, the identification of major compounds was performed in the present study. In this case, the EOs chemical composition was determined by gas-phase chromatography coupled to mass spectrometer (GC-MS), in a Shimadzu chromatograph GC-2010 Plus, GCMS-QP2010 Ultra equipped with an automatic sampler AOC model-20i (Souza et al. 2017a) and also by gas chromatography coupled to a flame ionization detector (GC-DIC), in a gas chromatograph Varian® CP-3380 equipped with DIC.

Initially, *L. sativae* larvae were obtained in melon leaves in Juazeiro (Bahia state), northeastern Brazil (9º36'35.4"S and 40º33'56.2"W). The species was reared in laboratory on cowpea plants [*Vigna unguiculata* (L.) Walp.] (Costa-Lima et al. 2010).

The melon plants used in the experiments were from the yellow type, "Gladial" variety. In a greenhouse, the seeds were weekly sown in a 200-cell tray containing Plantmax® commercial substrate. The seedlings were transplanted to cups (500 mL) containing sand and organic fertilizer (1:1). When the melon plants achieved two permanent completely expanded leaves (approximately 20 days after sowing), the plants were offered to *L. sativae* adults in breeding cages for 24 h. Afterwards, the plants were transferred to climatic chambers (25 ± 1 ºC, RH of 50 ± 20 % and 12 L:12 D photoperiod). After three days, the newly hatched larvae were counted under a stereoscopic microscope (40x) with transmitted light.

The treatments were evaluated with the following EOs: (i) *L. gracilis* leaves; (ii) *L. schaueriana* leaves; (iii) *C. sonderianus* leaves; (iv) *C. sonderianus* stem bark; (v) *C. conduplicatus* leaves; (vi) *C. conduplicatus* stem bark. The EOs concentration was 1,000 ppm, dissolved in distilled water with dimethyl sulfoxide (DMSO) 1 %. The control was

composed by 1 % DMSO. This solvent was chosen for not causing phytotoxicity, while others, as acetone, provoked leaf chlorosis and distortion.

The bioassay to assess the insecticide potential on the larval phase was adapted from Ferguson (2004). For each treatment, leaves with *L. sativae* larvae with less than 24 h were immersed for 5 s in the different solutions. After the immersion, the plants remained at room temperature for drying the leaves. Then, the plants were kept in climatic chambers (25 ± 1 ºC, RH of 50 ± 20 % and 12 L:12 D photoperiod).

The biological parameters evaluated were larval and pupal mortality and pupal duration. The larvae were daily evaluated using a stereoscopic microscope (40x). The newly formed pupae were transferred to Petri dishes (6 cm ø) coated with plastic film. In this step, the pupal viability and duration were calculated.

For the free-choice test, the EOs used were the same as in the previous experiment, with a 500 ppm concentration. The solutions were transferred to a prior compression sprayer (2 L). Melon plants with approximately 15 days after seeding were subjected to the respective treatments by spraying to run-off. The control treatment was composed of DMSO 1 %. After the evaporation of the moisture excess, the plants were kept in cages with screen mesh sides (40 cm x 39 cm for base and height of 50 cm). For the bioassay, two plants were placed in a cage, being one treated with EOs and the other only with DMSO 1 %. A honey solution (10 %) was provided as an adult food source. The treatments were kept in a controlled temperature room (25 ± 2 ºC, RH of 50 ± 20 % and 12 L:12 D photoperiod). Inside each cage, five *L. sativae* females were released (4-6 days old), which remained in contact with the plants for 24 h. The age range of the females was chosen based on the higher *L. sativae* oviposition rate period (Costa-Lima et al. 2010). After this period, the eggs and feeding punctures were counted under a stereoscopic microscope (90x) with transmitted light. In the no-choice bioassay, the method was similar to the one previously reported. However, in each cage, only one plant sprayed with a single treatment was maintained.

For the EOs insecticidal activity bioassay, the experimental design was completely randomized. Each insect was considered a replicate, ranging from 61 to 130 larvae and 59 to 117 pupae per treatment. Non-generalized linear models with quasi-binomial distribution for the larvae and pupae mortality data analysis were used. When there was a significant difference between the treatments, multiple comparisons (Tukey test; $p < 0.05$) were carried out by means of the function *glht multicomp* package, with adjustment of the p values. For the pupal duration, the averages and standard errors were defined by the Kaplan-Meier estimator (Kaplan & Meier 1958). Pairwise comparisons between the group levels were done using the log-rank test, with the *pairwise_survdiff()* function of the *survminer* package ($p < 0.05$).

The bioassays to evaluate the *L. sativae* oviposition and feeding had an experimental randomized block design. Each cage was considered a replicate, totalling 11 replicates for the free-choice test and six for the no-choice test. For the free-choice test data comparison, the F-test was applied at 5 % of significance. The no-choice data were subjected to analysis of variance and Tukey test ($p < 0.05$).

All analyses were performed using the R statistical software, version 3.5.2 (R Development Core Team 2018), and the graphic presentations using the SigmaPlot (2019) software, version 5.6 (Systat Software Inc., Chicago, II, USA).

RESULTS AND DISCUSSION

The *L. gracilis* and *L. schaueriana* leaves EOs presented an insecticide effect on the *L. sativae* larval stage, while the treatments composed by the *Croton* species did not differ from the control. The pupae originated from larvae exposed to *L. gracillis* EOs also had a lower viability. Considering the overall *L. sativae* mortality (larvae and pupae), *L. gracillis* and *L. schaueriana* reached a mean of 47.72 % and 45.71 %, respectively (Table 1).

This is the first report of *Lippia* EOs with insecticide activity on leafminer insects. Several authors have reported the activity of *L. gracillis* EOs on other arthropods, such as Lepidoptera larvae (Melo et al. 2018), mosquitoes (Maia et al. 2008) and ticks (Cruz et al. 2013). Regarding *L. schaueriana*, this was the first insecticidal activity record. Other *Lippia* species also have shown insecticidal effect on arthropods; for example, *Lippia alba* (Mill.) (Peixoto et al. 2015), *Lippia pedunculosa* (Hayek) (Nascimento et al. 2017) and *Lippia origanoides* (Kunth) (Castillo et al. 2017).

The main compounds present in the *L. gracillis* EOs were carvacrol (78.6 %) and thymol (6.3 %)

Table 1. Mortality (mean ± standard error) rate for *Liriomyza sativae* larvae, pupae and total (larvae + pupae), after the immersion of melon leaves with newly hatched larvae in essential oils solutions (1,000 ppm) and control (DMSO 1 %).

Treatment	Mortality (%)		
	Larvae	Pupae	Total
Lippia gracilis (leaf)	27.27 ± 2.90 a[1]	28.12 ± 5.66 a	47.72 ± 5.35 a
Lippia schaueriana (leaf)	30.00 ± 3.58 a	22.45 ± 6.02 ab	45.71 ± 5.99 a
Croton conduplicatus (stem)	15.60 ± 1.31 ab	21.00 ± 3.75 ab	33.33 ± 3.98 ab
Croton sonderianus (leaf)	14.67 ± 4.11 ab	21.87 ± 5.20 ab	34.66 ± 5.53 b
Croton sonderianus (stem)	12.21 ± 1.06 ab	8.69 ± 2.64 b	19.84 ± 3.49 b
Croton conduplicatus (leaf)	3.28 ± 2.29 b	16.95 ± 4.27 ab	19.67 ± 5.13 b
Control	10.20 ± 0.51 b	11.65 ± 1.71 b	20.66 ± 2.06 b

[1] Averages with different letters in the same column differ by the Tukey test ($p < 0.05$).

(Souza et al. 2017b). The *L. sativae* larval mortality is probably associated with a high concentration of carvacrol. This monoterpene is reported as toxic to agricultural and veterinary importance insects (Cruz et al. 2013, Park et al. 2017). Tong & Coats (2010) studied the carvacrol mode of action on *Musca domestica* L. and *Periplaneta americana* L. and showed the compound action as a positive allosteric modulator in the gamma-aminobutyric acid (GABA) receptor, the major inhibitory neurotransmitter in the central and peripheral insects nervous system. Thus, providing a chloride mediated by GABA increases the uptake and consequent inhibition in the nervous system and the death of insects.

For *L. schaueriana* leaves EOs, the chromatographic analysis identified as major compounds the piperitone oxide (73.5 %) and limonene (8.0 %) (Souza et al. 2018). For both the compounds, there are reports of toxicity on insects (Kim & Lee 2014, Momen et al. 2018). *Mentha microphylla* K. Koch EOs containing high piperitone oxide concentrations caused mortality in *Sitophilus oryzae* and *Tribolium castaneum* adults (Mohamed & Abdelgaleil 2008). Another species with a high level of the same compound, *Plectranthus mollis* (Aiton) Spreng. (Lamiaceae), showed a larvicidal activity on mosquitoes of medical importance (Kulkarni et al. 2013). Similarly to our study, the larvicidal activity has already been observed in other *Lippia* plants (i.e., *L. pedunculosa*), which presented deleterious effects on *Aedes aegypti* (L.) larvae (Nascimento et al. 2017).

Regarding *Croton* EOs, no larval mortality effect was observed on *L. sativae*. However, the pupae originated from the larvae pre-exposed to *C. conduplicatus* (leaves and stems) and *C. sonderianus* (stems) EOs presented a prolonged duration, if compared to the control ($p < 0.05$) (Table 2). Many EOs and their constituents may cause these effects, such as alteration in the development period (Park & Tak 2016). *Citrus limonum* (L.) *and Litsea cubeba* (Lour.) EOs are examples on *Tenebrio molitor* L., which resulted in prolonging the egg and larval period and shortening the beetle pupal phase (Wang et al. 2015). The authors identified β-pinene as one of the major compounds on the *C. limonum* EO. For *C. conduplicatus* stem bark EO, that caused elongation on the *L. sativae* pupal phase, β-pinene was also one of the main compounds (Oliveira et al. 2017).

In the present study, it was also possible to verify other effects on *L. sativae* in the EOs treatments, among these the darkened and dry pupae formation and adults with deformities. Probably, the EOs caused physiological changes that interfered in the insect metamorphosis. Similar alterations were observed in *M. domestica* pupae treated with monoterpenes, that showed a high incomplete emergence and adult malformation (Kumar et al. 2014).

In the free-choice test, the melon plants sprayed with *C. conduplicatus* leaves EO showed a

Table 2. *Liriomyza sativae* pupal stage duration (mean ± standard error), after the immersion of melon leaves with leafminer larvae in essential oils solutions (1,000 ppm) and control (DMSO 1 %) (25 ± 1 ºC; 50 ± 20 % of RH; 12 h).

Treatment	Pupa duration (days)
Croton conduplicatus (stem)	10.11 ± 0.08 a[1]
Croton conduplicatus (leaf)	9.69 ± 0.08 b
Croton sonderianus (stem)	9.61 ± 0.05 b
Lippia gracilis (leaf)	9.39 ± 0.08 bc
Croton sonderianus (leaf)	9.20 ± 0.11 c
Lippia schaueriana (leaf)	9.05 ± 0.12 c
Control	9.34 ± 0.04 c

[1] Means and standard errors were defined by the Kaplan-Meier estimator; thus, means without a common letter differ by the Log-rank test ($p < 0.05$).

2.7-fold lower number of eggs, when compared to the control (Figure 1A). For all the other treatments, no effect was observed on the *L. sativae* oviposition. The leafminer feeding preference was also not affected by the EOs spraying (Figure 1B).

In the no-choice test, the *C. conduplicatus* leaves also showed the best result, reducing in 20-fold the oviposition, if compared to the control. The *C. sonderianus* stem EO also caused a reduction in the number of eggs in 5.8-fold. Regarding the feeding punctures, four treatments with EOs presented a lower mean than the control: *C. conduplicatus* (leaf), *C. sonderianus* (stem and leaf) and *L. gracillis* (leaf) (Table 3).

In the bioassays with *L. sativae* adults, the *C. conduplicatus* leaves and *C. sonderianus* stems EOs probably provoked a repellent and/or deterrent action. The *Croton roxburghii* Balakr EO caused repellence to mosquitoes from the genus *Armigeres*, *Culex* and *Aedes* (Vongsombath et al. 2012). The *Croton malambo* (Karst) EO also caused repellence to the coleopteran *Tribolium castaneum* (Herbst.) (Jaramillo-Colorado et al. 2014).

The majority of the compounds present in the *C. conduplicatus* leaves EOs were 1-8-cineole (eucalyptol) (15.88 %), p-cymene (11.38 %) and spathulenol (11.23 %) (Almeida et al. 2014). Eucalyptol has been known for its insects repellence since the 1980's, from tests on *P. americana* (Scriven & Meloan 1984) and for *A. aegypti* (Klocke et al. 1987). The same compound caused deterrence on the beetle *T. castaneum* (Tripathi et al. 2001) and reduced the two-spotted spider mite oviposition (Roh et al. 2013). Thus, it is possible that eucalyptol also shows a repellent and/or deterrent action on *L. sativae*. Sensory cells located on the antennas must be related to stimulus detection, as already observed in *L. sativae*, in response to *Dolichandrone cauda-felina* (Hance) Benth. & Hook EO (Habita et al. 2007). For the mosquito *Culex quinquefasciatus* Say, a short antennal trichoid sensilla was identified, with a high sensitivity to eucalyptol (Syed & Leal 2008).

Table 3. Means (± standard error) for the number of eggs of *Liriomyza sativae* and feeding punctures in melon plants sprayed with essential oil solutions (500 ppm) and control (DMSO 1 %), in a no-choice test.

Treatment	Eggs	Feeding punctures
C. conduplicatus (leaf)	0.17 ± 0.17 a[1]	85.67 ± 17.52 a
C. sonderianus (stem)	5.83 ± 2.15 b	115.00 ± 26.69 ab
C. sonderianus (leaf)	13.5 ± 2.29 bc	213.67 ± 50.20 ab
L. gracilis (leaf)	16.33 ± 3.07 c	220.50 ± 24.79 b
C. conduplicatus (stem)	19.67 ± 4.01 c	325.33 ± 37.18 bc
L. schaueriana (leaf)	24.17 ± 1.54 c	328.33 ± 41.05 bc
Control	34.33 ± 8.27 c	413.33 ± 60.22 c

[1] Means followed by the same letter do not differ by the Tukey test ($p < 0.05$).

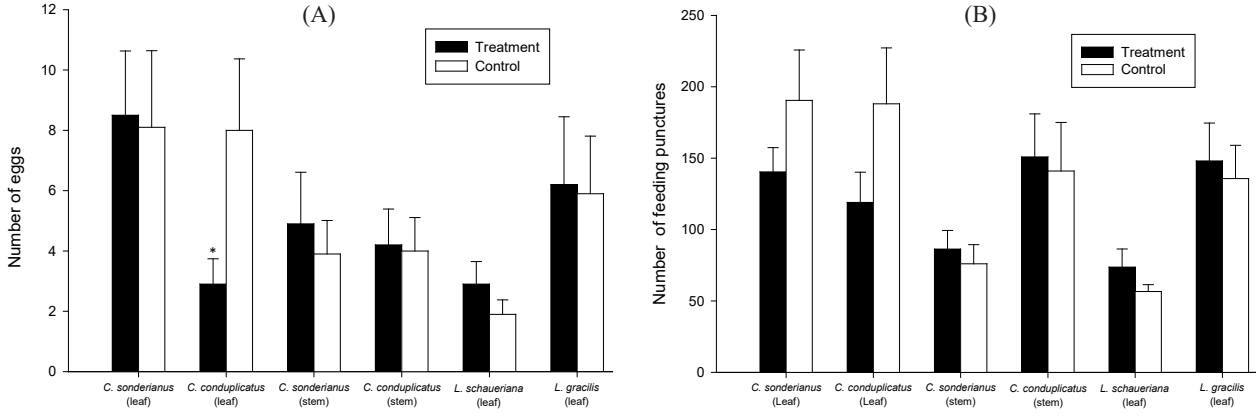

Figure 1. Means for number of *Liriomyza sativae* eggs (A) and feeding punctures (B) in melon plants treated with essential oils (500 ppm) and control (DMSO 1 %), after 24 h of exposure in a free-choice test. Bars above each column are the standard error of the mean. * Significant contrast "treatment *vs*. control" by the F-test at 5 % of probability.

The other two *C. conduplicatus* leaves EO main compounds, p-cymene and spathulenol (Almeida et al. 2014), are also reported as repellents for mosquitoes (Park et al. 2005). Probably all these main compounds are involved in the *L. sativae* oviposition drastic reduction observed in our results. For the *C. sonderianus* stems EO, which also caused a reduction in the *L. sativae* oviposition in the no-choice test, the main compound identified was α-pinene (42.14 %). This terpene is known as mosquito (Nerio et al. 2010) and *M. domestica* repellent (Haselton et al. 2015).

The present study brought new information on EOs of plants from the Caatinga biome, species with few studies or still not explored. A leafminer insect as a pest target must also be highlighted, considering the restricted reports of the EOs action over this insect guild. Nowadays, *Azadirachta indica* A. Juss. is the only botanical insecticide commercialized in Brazil to control leafminers (Agrofit 2019), showing the demand for new products in this area. Promising results, mainly related to the *L. sativae* oviposition reduction by *C. conduplicatus* leaves EOs, demonstrate the potential as a management strategy for this pest. Therefore, it is important the research continuity to detect the compounds involved in the effects, thus allowing to develop new products with a greater efficiency and less impact on the environment.

CONCLUSION

Essential oils from *Lippia gracilis* and *Lippia schaueriana* leaves show insecticide effect over *Liriomyza sativae* larvae, and the former also reduces the pupae viability, while the essential oil from *Croton conduplicatus* leaves reduces the *L. sativae* oviposition.

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