

Bioactivity of essential oils from *Croton grewioides* and its major compounds: toxicity to soybean looper *Chrysodeixis includens* and selectivity to the predatory stink bug *Podisus nigrispinus*

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Abstract

Natural biological control is a key factor that ensures the regulation of insect pest populations in agroecosystems. However, the indiscriminate use of pesticides has compromised this environmental service. Thus, the search for environmentally safe pesticides is an increasing requirement for sustainable food production. In this study, we analyzed the toxicity of essential oils from two accessions (CGR112 and CGR126) of *Croton grewoides* and its major compounds, methyl eugenol and eugenol, on the soybean pest *Chrysodeixis includens*. In addition, we investigated the sublethal effects of these compounds on the predatory bug *Podisus nigrispinus*, analyzing its developmental, reproduction and life table parameters. Essential oils and their major compounds were toxic to *C. includens* and *P. nigrispinus*. In general, the presence of eugenol made the essential oil more toxic to the pest and selective to the natural enemy. Eugenol was the most toxic compound for second instar larvae of *C. includens* at LD₅₀, followed by CGR126 essential oil from *C. grewoides* which was equally toxic and this compound at higher doses. The estimated lethal times for essential oils to cause mortality in 50% of the population of *C. includens* were less than 15 hours. There was selectivity of the essential oil of CGR126 accession of *C. grewoides* at lethal doses above 90%. Although the treatments showed little effect on the development of *P. nigrispinus*, body mass and reproductive parameters were negatively affected, with the exception of the essential oil of CGR126 accession of *C. grewoides*. The essential oil of *C. grewoides* may represent a promising source to be used in the synthesis of new insecticides, which are efficient against *C. includens* and at the same time are safer for the natural enemy *P. nigrispinus*.

1. Introduction

Biological control directly affects population density and effectiveness in controlling phytophagous (Desneux et al. 2007). However, the use of non-selective pesticides has reduced the chances of natural control, requiring increased doses and frequency of application of these pesticides in the field. Such indiscriminate use, contributes to the emergence of insect pest populations resistant to different classes of insecticides (Gonring et al. 1999; Bacci et al. 2001; Stacke et al. 2019). In addition, the inappropriate use of pesticides can cause accumulation of toxic residues in food, promote soil and water contamination and intoxication of rural producers (Dassanayake et al. 2021). Such problems are exacerbated, especially in large-scale agriculture, as is the case of soybean production in Brazil, which represents a large portion of the global supply of proteins and vegetable oils. The country stands out as the largest producer, with a productivity of around 125 million tons of soybeans in 2021 (Conab 2022). However, intensive soybean cultivation has caused outbreaks of pests that considerably affect its productivity and pesticide costs.

Among the soybean pest insects, the *Chrysodeixis includens* (Lepidoptera: Noctuidae), popularly known as soybean looper, this generalist insect can damage more than 174 plant species from 39 different botanical families, including beans (*Phaseolus* spp.), cotton (*Gossypium hirsutum*), tobacco (*Nicotiana* spp.), sunflower (*Helianthus* spp.), tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum*) (Specht et al. 2015). *C. includens* larvae have the habit of feeding on the medium and basal leaves of the

plants, which hinders the efficiency of insecticide application (Specht et al. 2015). Among the possible natural enemies of this pest, the predatory bug *P. nigrispinus* (Dallas 1851) (Hemiptera: Pentatomidae) stands out. This insect has the potential to control several species of pests of economic importance (Vivan et al. 2002; Vacari et al. 2007; Zanuncio et al. 2008; De Clercq et al. 1998). However, as a way of mitigating defoliation and damage caused by *C. includens*, farmers have opted for the chemical control method, which often results in the development of resistant insect populations (Stacke et al. 2020). Resistant pests demand higher doses of insecticides for their control, culminating in negative impacts on non-target organisms, thus compromising ecosystem services and human health (Aktar et al. 2009).

The adoption of integrated pest management (IPM) can contribute to mitigating the negative effects arising from the disorderly use of pesticides. Among the recommended tactics is the maintenance of populations of natural enemies, as well as the use of selective pesticides against these organisms. In this sense, essential oils (EOs) from plants can be a potential alternative to the use of organosynthetic insecticides (Melo et al. 2018), being in many cases considered promising for the synthesis of new insecticidal molecules (Bakkali et al. 2008; Isman et al. 2010). The EOs consist of products of secondary plant metabolism, which have evolved and chemically diversified in response to herbivory or environmental pressures. Such differential pressures, in time and space, have resulted in a great diversity of compounds, including chemical variations between individuals of the same species (Campos et al. 2015; Feitosa-Alcantara et al. 2017; Santos et al. 2017; Melo et al. 2018). This variety is related to different factors that can influence the biosynthesis of EOs, such as genetic variations, age, harvest time and abiotic effects (Cruz et al. 2014; Santos et al. 2016). Among the advantages of using plant EOs as biopesticides is their high volatility (low persistence in the environment) and the presence of a wide variety of compounds in their chemical constitution. Such chemical components are present in different proportions (eg major or minor compounds) (Blank et al. 2019) and interact in different ways (eg synergistic, additive or antagonistic) influencing the bioactivity of EOs (Cavar Zeljkovic and Maksimovic 2015).

Croton grewoides, popularly known as “canelinha-de-cheiro” or “canelinha”, is an endemic plant of the Caatinga biome, found in rocky regions and sandy soils in the states of the Northeast region (Silva et al. 2008). Studies show that the EOs obtained from *C. grewoides* can present a great diversification of compounds, thus representing important sources of bioactive substances for insect control (Farmer and Ryan 1990). Among such compounds, the following stand out: (*E*)-anethole, (*E*)-methyl isoeugenol, eugenol, methyl eugenol and methyl chavicol (Silva et al. 2008; Oliveira et al. 2021).

In this study, we evaluated the activity of two EOs accessions of *C. grewoides* and its major compounds considering the susceptibility of the herbivore *C. includens*, as well as the selectivity and the effects of these compounds on the biological and reproductive parameters of the natural enemy, the predatory stink bug *P. nigrispinus*.

2. Material And Methods

2.1 Rearing of insects

The population of *C. inncludens* used in the experiments was acquired from the company CL Empreendimentos Biológicos LTDA. The population was maintained at the Phytosanitary Clinic of the Universidade Federal de Sergipe (UFS), São Cristóvão-SE (10°54' S, 37°04' W, 7m de altitude), Brazil.

The larvae were individualized in 500 ml polypropylene pots, fed an artificial diet (Greene et al. 1976) and placed in a BOD growth chamber under controlled conditions of temperature and humidity ($27 \pm 0.5^{\circ}\text{C}$, RH of $70 \pm 10\%$ and photoperiod of 12 hours). Upon reaching the pupal stage, they were sexed and kept in cylindrical plastic cages (30 cm x 30 cm x 30 cm) lined with bond paper, used as a substrate for oviposition. The cages were sealed with organza fabric. Eight couples were added to each cage, fed daily with a 20% sugar solution.

The population of *P. nigrispinus* used was obtained from breeding maintained at the Laboratory of Forest Entomology (LEFLO) of the Universidade Federal de Sergipe (UFS). The insects were kept in 500 ml polypropylene pots, fed *ad libitum* with 7th instar larvae of *Tenebrio molitor*. The eggs deposited in the cages were collected daily with the aid of hydrophilic cotton and transferred to Petri dishes (9 x 1.5 cm) containing cotton moistened with water. *T. molitor* larvae used to feed *P. nigrispinus* were fed with soybean meal and kept in plastic trays measuring 24 x 20 x 15 cm.

2.2 Obtaining the compounds and chemical analysis of the EOs

The EOs of *C. grewioides* were obtained from accessions CGR112 and CGR126 that are kept in the active germoplasm bank of the Universidade Federal de Sergipe (UFS) – located on the experimental farm – Campus Rural da UFS, São Cristóvão, SE, Brazil (10°55 S, 37°11' W, 18 m de altitude).

Initially, the leaves removed from the plants were dried in ovens at 40°C for 5 days. The EOs were extracted by hydrodistillation in a modified cleverger apparatus using 70 g of samples of dried leaves for 120 min from boiling at 100°C . The EO obtained from each sample was stored at -20°C until the chemical composition was analyzed. To identify and quantify the compounds, analyzes of the EOs were performed (for more details, see Oliveira et al. 2021). The major compounds methyl eugenol and eugenol, found in the EOs of the accessions studied, were purchased from the company SIGMA-ALDRICH.

2.3 Bioassays

The bioassays were performed at the Phytosanitary Clinic, Universidade Federal de Sergipe (UFS), São Cristóvão-SE.

The treatment used were the EOs of accessions CGR112 and CGR126 of *C. grewioides* and the major compounds methyl eugenol and eugenol. The solvent used to dilute the compounds was acetone (Panreac, UV-IR-HPLC-GPC PAIACS, 99,9%). The treatments were applied topically to the prothoracic region of each insect with the aid of a microsyringe (Hamilton®, Reno, NV, USA), with 0.5 μL for *C.*

inclusens and 1 μ L for *P. nigrispinus*. In the control, only acetone was applied. Preliminary tests have indicated that acetone does not affect insect survival, development and reproduction.

For the determination of insect mass, 50 second-instar larvae of *C. inclusens* and 50 third-nymphs of *P. nigrispinus* were individually weighed on a precision balance (Shimadzu, AUW220D).

2.3.1 Toxicity and selectivity

Preliminary tests were performed with three doses (1, 5 and 10 μ g of the substances/mg of insect) of each treatment. From these tests, at least six doses were determined to plot the dose-mortality curves. The experimental design used was completely randomized, with four replications per treatment.

The treated *C. inclusens* larvae were placed in cell culture plates (Global Trade Technology, Monte Alto, SP, Brazil) (24 holes) lined with moistened filter paper, containing bean leaf ($\cong$ 6 cm²) for feeding. Each experimental unit consisted of 12 insects.

Before treatment applications, *P. nigrispinus* nymphs were left in a freezer for 90 s to reduce activity. Preliminary tests indicated that this time spent in the freezer does not affect the survival of the insects. Treated *P. nigrispinus* nymphs were transferred to Petri dishes (Global Trade Technology, Monte Alto, SP, Brazil) (9 x 1.5 cm) containing moistened hydrophilic cotton and 7th instar larvae of *T. molitor* were provided *ad libitum* as a food source. Each experimental unit consisted of 10 insects.

Cell culture dishes containing the larvae and Petri dishes containing nymphs were kept in a BOD growth chamber under controlled conditions (Biotech®, Piracicaba, SP, Brazil) ($27 \pm 0.5^\circ\text{C}$, RH $70 \pm 10\%$ and photoperiod of 12 hours). Insect mortality was verified 72h after setting up the bioassays. Insect that did not move after being stimulated with a soft-bristled brush were considered dead.

Dose-response curves were used to determine the toxicity and selectivity of the compounds in favor of the predatory bug.

2.3.2 Lethal time

In the bioassays with *C. inclusens* and *P. nigrispinus*, the larvae and nymphs were submitted to the application of the LD₅₀ of the treatments, being later conditioned in the same way as in the toxicity and selectivity bioassays. The experimental design used was completely randomized, with seven and ten replications per treatment, for *C. inclusens* and *P. nigrispinus*, respectively. LD₅₀ was used to determine survival curves and lethal time to kill 50% of the population (LT₅₀) as it is a standard of control efficiency in Brazil.

The first evaluations were carried out 10 and 30 min after the assembly of the bioassays, later evaluations were carried out every 2h for 24h, followed by 4h intervals in the following 24h, and finally evaluations were carried out every 12h until 120h.

2.3.3 Biological parameters

To assess the sublethal effects on biological parameters (development, longevity and mass) of *P. nigrispinus*, third instar nymphs were topically treated with 1 µl of the LD₃₀ of each treatment. Seven cohorts with 10 replicates each were used. The nymphs were kept individually in a Petri dish (Global Trade Technology, Monte Alto, SP, Brazil) (9 x 1.5 cm) containing moistened cotton. The plates were kept in an acclimatized environment (27 ± 0.5°C, RH 70% and photoperiod of 12 hours). Nymphs were fed *ad libitum* with *T. molitor* larvae. Mortality and durations at each instar were assessed daily.

The insects that reached the adult stage had their mass determined, were sexed and isolated during a period of 3 days to acquire sexual maturity. Ten couples/treatment were formed, which were kept in plastic pots (500 ml) and fed *ad libitum* with *T. molitor* larvae. After the formation of the couples, the periods of pre-oviposition, oviposition, number of eggs per female, number of egg masses per female, number of eggs per egg mass, number of nymphs per female, number of nymphs per mass of eggs, egg incubation period, egg viability and longevity off emales and males were evaluated.

2.3.4 Life table parameters

The life table parameters were calculated taking into account the survival and reproduction of *P. nigrispinus* exposed to the treatments. For this, the average age of the insects ($\bar{x}$), the specific survival rate (l_x) and the specific fertility (m_x) were calculated.

The life table was constituted by the net reproductive rate [$R_0 = \sum (l_x.m_x)$], the intrinsic rate of increase [$r_m = \ln(R_0)/T$], average generation time [$T = (\sum x.l_x.m_x)/(\sum l_x.m_x)$], population doubling time [$TD = \ln(2)/r_m$] and finite rate of increase [$\lambda = e^{r_m}$].

2.4 Statistical analysis

Toxicity data obtained for *C. includens* and *P. nigrispinus* were submitted to Probit analysis to determine dose-mortality curves using the PROC PROBIT procedure (SAS Institute 2008). The curves were obtained with a probability of acceptance of the null hypothesis (that the data have a Probit distribution) greater than 0.05 by the χ^2 test. From the curves, the LDs (1 to 99) with their respective 95% confidence intervals (95%CI) were obtained. Curves and LDs were compared using the criterion of non-overlapping confidence intervals (95%CI) with the origin of the interval.

Survival analyzes were performed using Kaplan-Meier estimators using the Log-Rank test (SigmaPlot, version 12.5). Through this analysis, survival curves and lethal times capable of causing mortality in 50% of the individuals (LT₅₀) (*C. includens* or *P. nigrispinus*) were obtained, with their respective confidence intervals to 95% (IC_{95%}). The curves were compared by the Holm-Sidak multiple comparison method at 5% probability (SigmaPlot, 12.5 version). The LT_{50s} were compared using the criterion of non-overlapping confidence intervals (95%CI) with the origin of the interval.

The life table parameters were estimated using the jackknife technique with the SAS statistical program (Maia et al. 2000). The data on adult longevity, female and male mass, reproductive parameters and life table parameters were initially submitted to the Kolmogorov-Smirnov test to verify the normality and heterogeneity of the data (SigmaPlot, 12.5 version). The variables that showed normality were submitted to analysis of variance followed by Dunnett's test ($P < 0.05$) for verify differences between treatments with the control, using ANOVA analysis of variance, with Dunnett's (SigmaPlot, 12.5 version). The variables that did not show normality were submitted to non-parametric analysis of variance of Kruskal-Wallis followed by the method of multiple comparison of Dunn's to compare the effect of the treatments with the control, using the ANOVA analysis of variance, with Dunn's (SigmaPlot, 12.5 version).

3. Results

3.1 Characterization of essential oils

Eleven compounds were identified in the EOs of both accessions of *C. grewioides*. The major compounds found in the EOs of the accessions were methyl eugenol (85.6%) for CGR112 and methyl eugenol (46%) and eugenol (42.7%) for CGR126. The EO contents were 4.1 and 2% for accessions CGR112 and CGR126, respectively (Fig. 1).

3.2 Toxicity and selectivity

The EOs obtained from *C. grewioides* accessions CGR112 and CGR126 and their major compounds methyl eugenol and eugenol were toxic to *C. includens* larvae and *P. nigrispinus* nymphs. The LD_{50} obtained for the treatments ranged from 4.65 to 8.82 $\mu\text{g}/\text{mg}$ for *C. includens* and from 1.22 to 4.45 $\mu\text{g}/\text{mg}$ for *P. nigrispinus*. At higher doses, EO from accession CGR126 and the compound eugenol were more toxic to *C. includens* than EO from accession CGR112 and its major compound methyl eugenol (Fig. 2).

The EO of *C. grewioides* accession CGR126 was selective to the predatory bug *P. nigrispinus* at doses higher than LD_{90} . Doses needed to kill between 65 and 90% of populations caused similar effects between *C. includens* and *P. nigrispinus* (Fig. 2b).

Likewise, the compound eugenol caused similar mortality among these species at lethal doses higher than those necessary to cause mortalities in 65% of the populations (LD_{65}) (Fig. 2d). At the standard control dose (LD_{90}), the EO of the CGR126 accession of *C. grewioides* (*C. includens* = 11.17 $\mu\text{g}/\text{mg}$; *P. nigrispinus* = 17.06 $\mu\text{g}/\text{mg}$) and the eugenol compound (*C. includens* = 9.73 $\mu\text{g}/\text{mg}$; *P. nigrispinus* = 10.90 $\mu\text{g}/\text{mg}$) were 1.5 and 1.1 fold more toxic to the pest than to the predatory bug (Fig. 2b,d).

The EO of *C. grewioides* accession CGR112 (LD_{50} : *C. includens* = 8.14 $\mu\text{g}/\text{mg}$; *P. nigrispinus* = 1.44 $\mu\text{g}/\text{mg}$) and its major compound methyl eugenol (LD_{50} : *C. includens* = 8.82 $\mu\text{g}/\text{mg}$; *P. nigrispinus* = 1.22 $\mu\text{g}/\text{mg}$) were not selective for the predator *P. nigrispinus*. The doses needed to kill 50% of the

populations of *C. includens* were 5.6 and 7.2 times higher than the doses used for *P. nigrispinus*, respectively (Fig. 2a,c).

3.3 Survival

Survival of *C. includens* larvae and *P. nigrispinus* nymphs exposed to LD₉₀s of treatments was significantly reduced over time (*C. includens*: Log-Rank test: $X^2 = 288.42$; $P < 0.001$ and *P. nigrispinus*: Log-Rank test: $X^2 = 230.96$; $P < 0.001$) (Fig. 3a,c).

There was no difference between the survival curves of *C. includens* larvae exposed to the major compounds methyl eugenol and eugenol ($P = 0.26$) (Fig. 3a). Likewise, the survival curves of *P. nigrispinus* nymphs exposed to the EO of the accession CGR112 of *C. grewiooides* and its major compound methyl eugenol did not differ ($P = 0.14$), as well as the curves of the EO of the accession CGR126 of *C. grewiooides* and the compound eugenol ($P = 0.73$) (Fig. 3c).

C. includens larvae showed a rapid reduction in survival when exposed to the EO of *C. grewiooides* accession CGR126 (LT₅₀ = 1.9 h; CI_{95%} = 1.2–2.6). In less than 11h, all individuals died (Fig. 3a,b). The EO of *C. grewiooides* accession CGR112 also caused a rapid reduction at the beginning, however some larvae remained alive until 104h (LT₅₀ = 14.6 h; CI_{95%} = 8–21.3) (Fig. 3a,b). The major compounds methyl eugenol and eugenol were slower to cause *C. includens* larvae mortality, with mean lethal times of 62.9h (CI_{95%} = 50.3–75.4) and 71.4h (CI_{95%} = 58.8–84.1), respectively (Fig. 3a,b).

For *P. nigrispinus*, the EO of accession CGR112 of *C. grewiooides* caused faster mortality (LT₅₀ = 31.5 h; CI_{95%} = 27.5–35.6), followed by its major compound methyl eugenol (LT₅₀ = 37.3h; CI_{95%} = 33.6–41) (Fig. 3c,d). On the other hand, the EO of the CGR126 accession of *C. grewiooides* (LT₅₀ = 48.2h; CI_{95%} = 42.5–60) and eugenol (LT₅₀ = 51.1 h; CI_{95%} = 44.7–57.5) caused slower mortality (Fig. 3c,d).

3.4 Development and longevity of *P. nigrispinus*

Before *P. nigrispinus* nymphs were exposed to the compounds (LD₃₀), the developmental times of the cohorts did not differ between egg stages ($H = 0.0$; $P = 1.00$); 1st instar nymph ($H = 0.382$; $P = 0.984$) and 2nd instar nymph ($H = 3.692$; $P = 0.449$). These stages had a mean duration of 4 ± 0 , 2.3 ± 0.0495 and 3.68 ± 0.0435 days, respectively (Fig. 4).

The durations of the 4th and 5th instar and the total nymphal phase of *P. nigrispinus* differed significantly between treatments (4th instar: $H = 17.94$; $P = 0.001$; 5th instar: $H = 12.54$; $P = 0.014$; total of the nymphal stage: $H = 9.722$; $P = 0.045$). The methyl eugenol and eugenol compounds increased the duration of the 4th instar by 0.59 and 0.53 days compared to the control. Likewise, the duration of the 5th instar increased by 0.62 days for individuals who were exposed to EO from the CGR126 accession of *C. grewiooides*. These variations resulted in an increase in the mean duration of the 1-day nymphal phase when *P. nigrispinus* nymphs were exposed only to the methyl eugenol compound (Fig. 4).

There was no effect of the compounds on the duration of 3rd instar nymphs ($H = 5.627$; $P = 0.229$) and on the longevity of females ($H = 2.962$; $P = 0.564$) and males ($H = 4.611$; $P = 0.330$) of *P. nigrispinus*. On average, the 3rd instar lasted 3.18 ± 0.0434 days and the longevity of females and males was 25.26 ± 1.68 days and 26.62 ± 1.67 days, respectively (Fig. 4).

3.5 *P. nigrispinus* mass

The mass of females and males of *P. nigrispinus* submitted to LD_{30s} reduced significantly when compared to the control ($P < 0.001$). On average, the compounds reduced the mass of females and males by 30 and 44%, respectively (Fig. 5).

3.6 Reproductives parameters

The pre-oviposition period ($H = 19.95$; $P < 0.001$), the number of eggs per female ($F = 2.82$; $P = 0.036$), the number of nymphs per female ($F = 2.72$; $P = 0.041$) and egg incubation ($H = 68.39$; $P < 0.001$) of *P. nigrispinus* differed from the control. The other reproductive parameters did not change with the application of the compounds ($P > 0.05$) (Table 1). The pre-oviposition period and egg incubation increased when the predatory stink bug was exposed to eugenol and EO from *C. grewioides* accession CGR126, respectively. The numbers of eggs and nymphs per female reduced when the insects were treated with methyl eugenol (Table 1).

Table 1

Reproductive parameters of *Podisus nigrispinus* after exposure of 3rd instar nymphs to LD₃₀s of essential oils from accessions CGR112 and CGR126 of *Croton grewoides* and its major compounds methyl eugenol and eugenol.

Reproductive parameters	Treatments				
	Control	CGR112	CGR126	Methyl eugenol	Eugenol
Pre-oviposition period (days) ^a	2.8 (± 0.20)	2.7 (± 0.26)	2.6 (± 0.27)	4.4 (± 0.65)	5.3 * (± 1.25)
Oviposition period (days) ^{a n.s.}	17.3 (± 2.48)	20.9 (± 3.16)	16.3 (± 3.51)	18.3 (± 2.88)	21.9 (± 4.03)
Number of eggs/ female ^b	189.9 (± 5.71)	183.8 (± 16.13)	151.8 (± 22.29)	126.9 * (± 15.83)	135.3 (± 19.52)
Number of egg masses/ female ^{b n.s.}	12.0 (± 1.07)	12.4 (± 1.46)	10.5 (± 1.62)	8.7 (± 0.78)	9.3 (± 1.16)
Number of eggs/ egg dough ^{b n.s.}	16.76 (± 1.21)	15.27 (± 0.76)	14.76 (± 0.79)	14.38 (± 0.97)	14.66 (± 0.81)
Number of nymphs / female ^b	163.5 (± 5.71)	160.4 (± 14.33)	132.5 (± 19.38)	109.9 * (± 14.27)	116.6 (± 17.15)
Number of nymphs/ egg dough ^{b n.s.}	14.42 (± 1.05)	13.30 (± 0.66)	12.91 (± 0.71)	12.42 (± 0.89)	12.61 (± 0.68)
Incubation of eggs (days) ^a	4.06 (± 0.03)	4.04 (± 0.02)	4.40 * (± 0.06)	4.08 (± 0.04)	4.04 (± 0.03)

^a Means (± standard error) with asterisk differ from control by Dunn's non-parametric multiple comparison method at $P < 0.05$. ^b Means (± standard error) with asterisk differ from control by Dunnett's test at $P < 0.05$.

n.s. not significant at 5% probability.

Reproductive parameters	Treatments				
	Control	CGR112	CGR126	Methyl eugenol	Eugenol
Viability of eggs	86.01	87.12	87.39	86.25	86.12
(%) ^b n.s.	(± 0.81)	(± 1.01)	(± 0.52)	(± 0.76)	(± 0.64)
^a Means (± standard error) with asterisk differ from control by Dunn's non-parametric multiple comparison method at $P < 0.05$. ^b Means (± standard error) with asterisk differ from control by Dunnett's test at $P < 0.05$.					
n.s. not significant at 5% probability.					

3.7 Demographic parameters

All demographic parameters of *P. nigrispinus* were affected by the application of the compounds (Table 2). The net reproductive rate (R_0) ($F_{4;49} = 2.80$; $P = 0.037$) was reduced with the exposure of the nymphs to the methyl eugenol compound. Likewise, the intrinsic rate of increase (r_m) ($F_{4;49} = 9.39$; $P < 0.001$) and the finite rate of increase (λ) ($F_{4;49} = 9.46$; $P < 0.011$) also decreased with the exposure of nymphs to methyl eugenol and eugenol compounds. On the other hand, the mean generation time (T) ($H = 13.88$; $P = 0.008$) for nymphs exposed to eugenol and the doubling time (DT) ($H = 22.85$; $P < 0.011$) of exposed nymphs to methyl eugenol and eugenol increased (Table 2).

Table 2

Life table parameters of *Podisus nigrispinus* after exposure of 3rd instar nymphs to LD₃₀s of essential oils from accessions CGR112 and CGR126 of *Croton grewoides* and its major compounds methyl eugenol and eugenol.

Life table parameter	Treatments				
	Control	CGR112	CGR126	Methyl eugenol	Eugenol
Net reproductive rate (R_0) (offspring/individual) ^a	80.03 (± 2.41)	69.58 (± 6.11)	60.72 (± 8.92)	51.67* (± 6.45)	56.05 (± 8.08)
Intrinsic rate of increase (r_m) (day ⁻¹) ^a	0.143 (± 0.002)	0.132 (± 0.004)	0.131 (± 0.005)	0.112* (± 0.005)	0.106* (± 0.007)
Mean generation time (T) (day ⁻¹) ^b	30.67 (± 0.65)	32.06 (± 1.34)	30.88 (± 1.57)	35.06 (± 1.62)	38.16* (± 2.57)
Doubling time (DT) (day ⁻¹) ^b	4.85 (± 0.09)	5.28 (± 0.17)	5.33 (± 0.20)	6.33* (± 0.36)	6.78* (± 0.49)
Finite rate of increase (λ) (day ⁻¹) ^a	1.15 (± 0.003)	1.14 (± 0.005)	1.14 (± 0.006)	1.12* (± 0.006)	1.11* (± 0.008)
^a Means (± standard error) with asterisk differ from control by Dunn's non-parametric multiple comparison method at $P < 0.05$. ^b Means (± standard error) with asterisk differ from control by Dunnett's test at $P < 0.05$.					

4. Discussion

In this study we evaluated the lethal effects of EOs from two accessions of *C. grewoides* and its major compounds methyl eugenol and eugenol on the insect pest *C. includens*, as well as the selectivity and sublethal effects of these compounds on the natural enemy *P. nigrispinus*. The OEs of the accessions showed distinct bioactivity. In general, the EO of the CGR126 accession was more toxic to pest and selective to the natural enemy.

The distinct toxicity of EOs can be due to differential interactions between the physicochemical properties of the compounds and the physiology and metabolism of the insects, affecting the rate of penetration, metabolism, excretion and action at the sites of action of the compounds (Gerolt 1983; Resende et al. 2016). Although biological activity is often attributed to the major compounds, EOs are made up of a complex mixture of compounds that can result in interactions that affect their activity (Atanasova and

Leather 2018; Isman 2016; Regnault-Roger et al. 2012). Both EO of accessions CGR112 and CGR 126 of *C. grewioides* present methyl eugenol as the major compound, however, only the EO of accession CGR126 has eugenol (42%) in its constitution. Such difference seems to be responsible for the distinct bioactivity presented by the EOs. In general, the presence of eugenol seems to have made the EO more toxic to the pest and selective to the natural enemy. In fact, eugenol was the compound that showed the greatest toxicity to *C. includens* at LD₅₀, followed by the EO from *C. grewioides* accession CGR126, which was equally toxic to this compound at higher doses (> LD₈₅). Eugenol has contact insecticidal action promoting rapid mortality in numerous insect pest species (Baker and Grant 2018; Dayan et al. 2009; Enan 2005; González Armijos et al. 2019). The action of contact of eugenol has been attributed to its rapid penetration into the cuticle of insects, having as main targets the octopamine receptors and transient receptor potential (TRP) channels, which can alter the functioning of the insect nervous system (Baker and Grant 2018; Enan 2005; Price and Berry 2006).

On the other hand, the methyl eugenol and the EO from the CGR112 accession of *C. grewioides*, which has this compound as the majority, were less toxic to the second instar larvae of *C. includens*. At this pest is generalist, it can have contact with methyl eugenol throughout its life history. This compound is present in more than 60 plant families and is possibly related to attraction of insects for pollination, as it can be released as a component of floral fragrance (Tan et al. 2012).

The LD₅₀ values obtained for *C. includens*, in general, were higher when compared to the LD₅₀ values obtained for *P. nigrispinus*, possibly due to the fact that the pest is naturally more exposed to the compounds than its natural enemy, the which can trigger resistance mechanisms on the part of the herbivore. The EO of *C. grewioides* accession CGR112 and methyl eugenol did not show selectivity, being more toxic to the natural enemy than to the pest (Fig. 2a,c). However, access CGR126 was selective and eugenol was equally toxic to the pest and the natural enemy, when in high doses (Fig. 2). In these cases, *C. includens* mortality increased rapidly with small variations in doses, which indicates a more homogeneous population response to these compounds. These results indicate that small variations in the doses of these compounds can cause greater variations in pest mortality than in the natural enemy (Bacci et al. 2001; Bacci et al. 2009). Above LD₉₀, the EO of the CGR126 accession of *C. grewioides* was more toxic to the pest than to the natural enemy (Fig. 2). The efficiency standard recommended in Brazil coincides precisely with such this doses (> LD₈₀), which would guarantee greater safety to the predator. Still, at this dosage, the EO of the CGR126 accession caused rapid mortality of the pest and slow mortality of the predatory bug, showing high efficiency and selectivity (Fig. 3). The slower effect may allow the predator time to metabolize compounds via detoxifying enzymes. It should be noted that, considering the development of pesticides based on these compounds, in addition to the physiological selectivity observed here, ecological measures can be adopted to increase selectivity, including the form and time of application.

All compounds tested showed little effect on the developmental cycle of *P. nigrispinus*. Third instar nymphs submitted to subdoses (LD₃₀) of methyl eugenol had a longer total duration of the nymphal

period, but this result was only one day, which does not interfere with the predators life cycle. On the other hand, our results show a significant reduction in the body mass of females and males of *P. nigrispinus* exposed to the compounds when compared to the control. Possibly, the insects submitted to the treatments spent energy to get rid of the compounds, thus reducing the feeding, with consequent loss of body mass. Since smaller males may prefer larger females (eg, provide a greater number of offspring) (Pereira et al. 2017), this could interfere with the reproductive potential. In fact, methyl eugenol caused a reduction in the number of eggs and nymphs per female, in addition to a decrease in the net reproductive rate, intrinsic rate of increase and finite rate of increase, further increasing the time for population doubling. On the other hand, this result does not seem to be attributed only to the reduction in body mass of females, since the other compounds tested also reduced the mass of insects and did not necessarily affect reproductive and life table parameters. Although eugenol had little effect on reproductive parameters, increasing only the pre-oviposition period, this compound affected the life table parameters of *P. nigrispinus*, reducing the intrinsic rate of increase and the finite rate of increase, increasing the generation time and duplication. Studies show that insecticides in general, including essential oils, can have effects on the morphology and histochemistry of the testes and ovarioles of insects, thus affecting their demographic potential (Santos et al. 2021, Alves et al. 2014).

In conclusion, our results show that despite all treatments being toxic against *C. includens*, only the EO from the accession CGR126 of *C. grewioides* shows promise for future use in sustainable management because it is more efficient and safer to the predatory bug *P. nigrispinus*. Compounds isolated from this EO (eg, eugenol) may also be promising, provided that ecological selectivity is observed.

Conclusions

In the presente study we evaluated the toxicity and the effect of essential oils obtained from accessions of the plant species *Croton grewioides* (CGR 112 and CGR126), and its major compounds (methyl eugenol and eugenol) on the survival of the pest *Chrysodeixis includens* and its natural enemy the predatory bug *Podisus nigrispinus*. In addition, the sublethal effects caused by the treatments on the reproductive and demographic parameters of the natural enemy were verified. The results showed that there was a difference in the response of the treatments between the pest and the natural enemy. The essential oil of CGR126 accession and its major compound eugenol proved to be more selective to the natural enemy at higher doses. Although the treatments caused a reduction in the weight of insects (males and females), and affected reproductive and demographic parameters and life table, only methyl eugenol showed significant deleterious effects on the stink bug *P. nigrispinus*. In this way, the botanical insecticides obtained from essential oils of accessions of *C. grewioides* proved to be potential sources of insecticides for the control of *C. includens*, and can be used as imortant tools in the integrated management of this pest.

Declarations

Ethical approval and consent to participate

Not applicable.

Consent to publish

All authors have given their consent to publish this research article.

Authors contributions

All authors contributed to the study conception and design. **Natielli Conceição Santos** was responsible for data curation, investigation, writing – original draft. **Jefferson Elias da Silva** was responsible for investigation, writing – review & editing. **Ane Caroline Celestino Santos** was responsible for data curation and investigation. **Jaciele de Oliveira Dantas** was responsible for data curation and investigation. **Swamy Rocha Siqueira Abreu Tavares** was responsible for data curation and investigation. **Valfran Silva Andrade** was responsible for data curation. **Sara Dayan da Silva Oliveira** was responsible for resource and investigation. **Arie Fitzgerald Blank** was responsible for resource and investigation. **Ana Paula Albano Araújo** was responsible for writing-review & editing, visualization. **Leandro Bacci** was responsible for conceptualization, methodology, validation, formal analysis, writing-review & editing, supervision. The final version of the manuscript was reviewed and approved by all authors.

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Conflict of interests

The authors declare no competing interests.

Availability of data and materials

The data and materials used to support the findings of this study are available from the corresponding author upon request.

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Figures

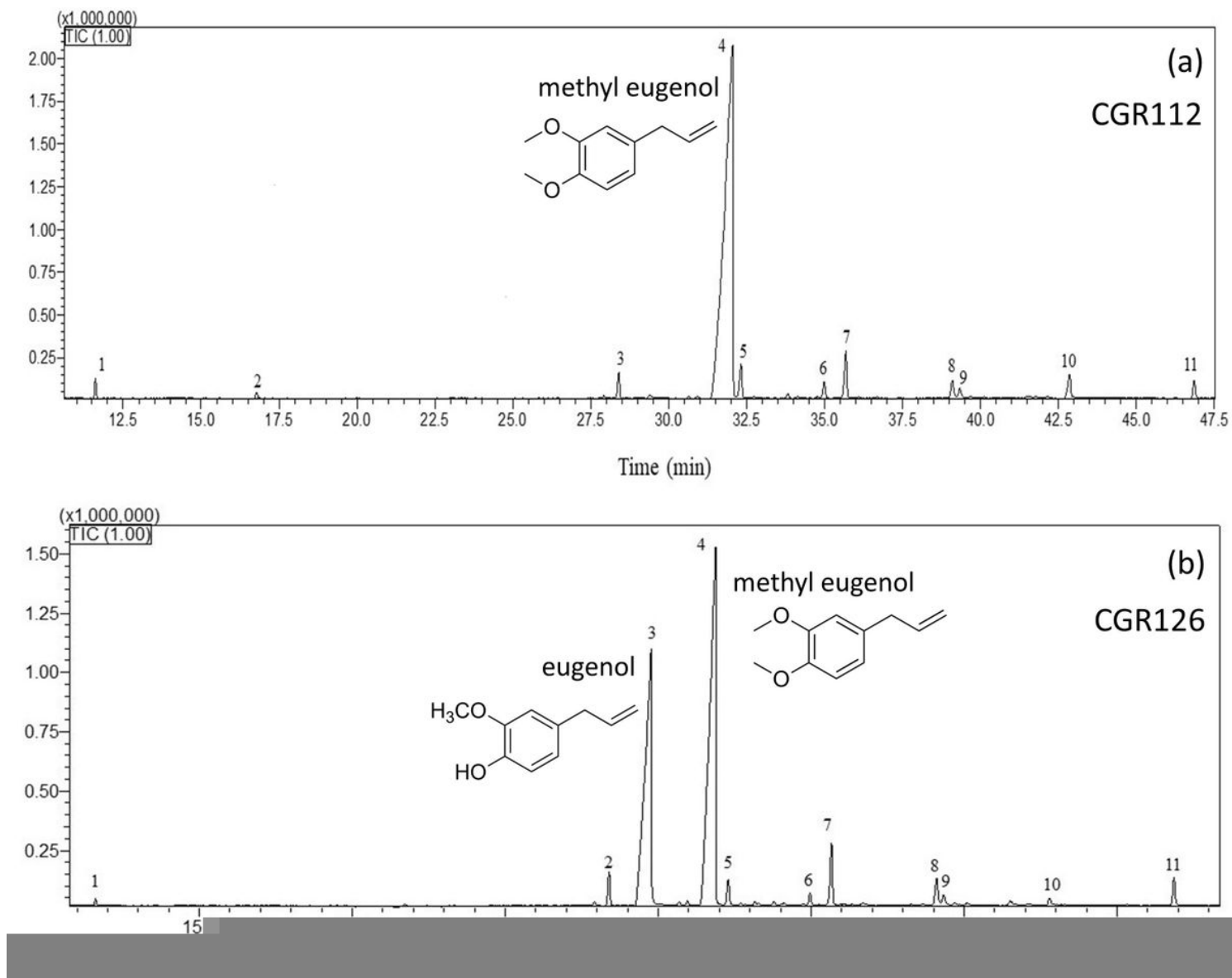


Figure 1

Chromatograms of essential oils from accessions CGR112 (a) and CGR126 (b) of *Croton grewiooides*.

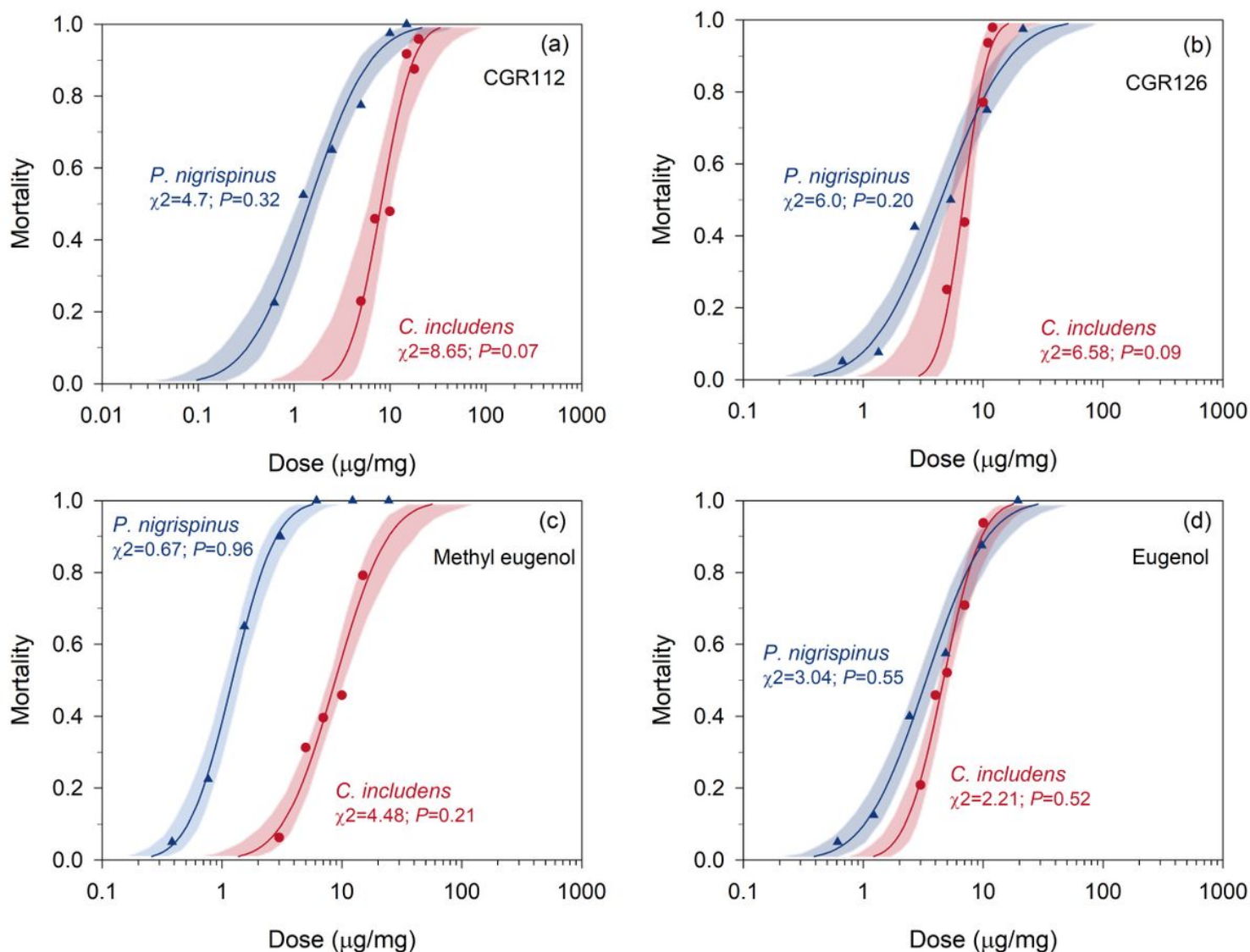


Figure 2

Toxicity and selectivity by topical application of essential oils from accessions CGR112 (a) and CGR126 (b) of *Croton grewoides* and its major compounds methyl eugenol (c) and eugenol (d) on 2nd instar larvae of *Chrysodeixis includens* (red) and nymphs 3rd instar of *Podisus nigrispinus* (blue). Lethal doses (log scale) were estimated based on dose-response bioassays using Probit analysis. Shaded areas represent 95% confidence intervals. Circles and triangles indicate the average mortalities observed in the bioassays for *C. includens* and *P. nigrispinus*, respectively. N = 240 for each treatment and species.

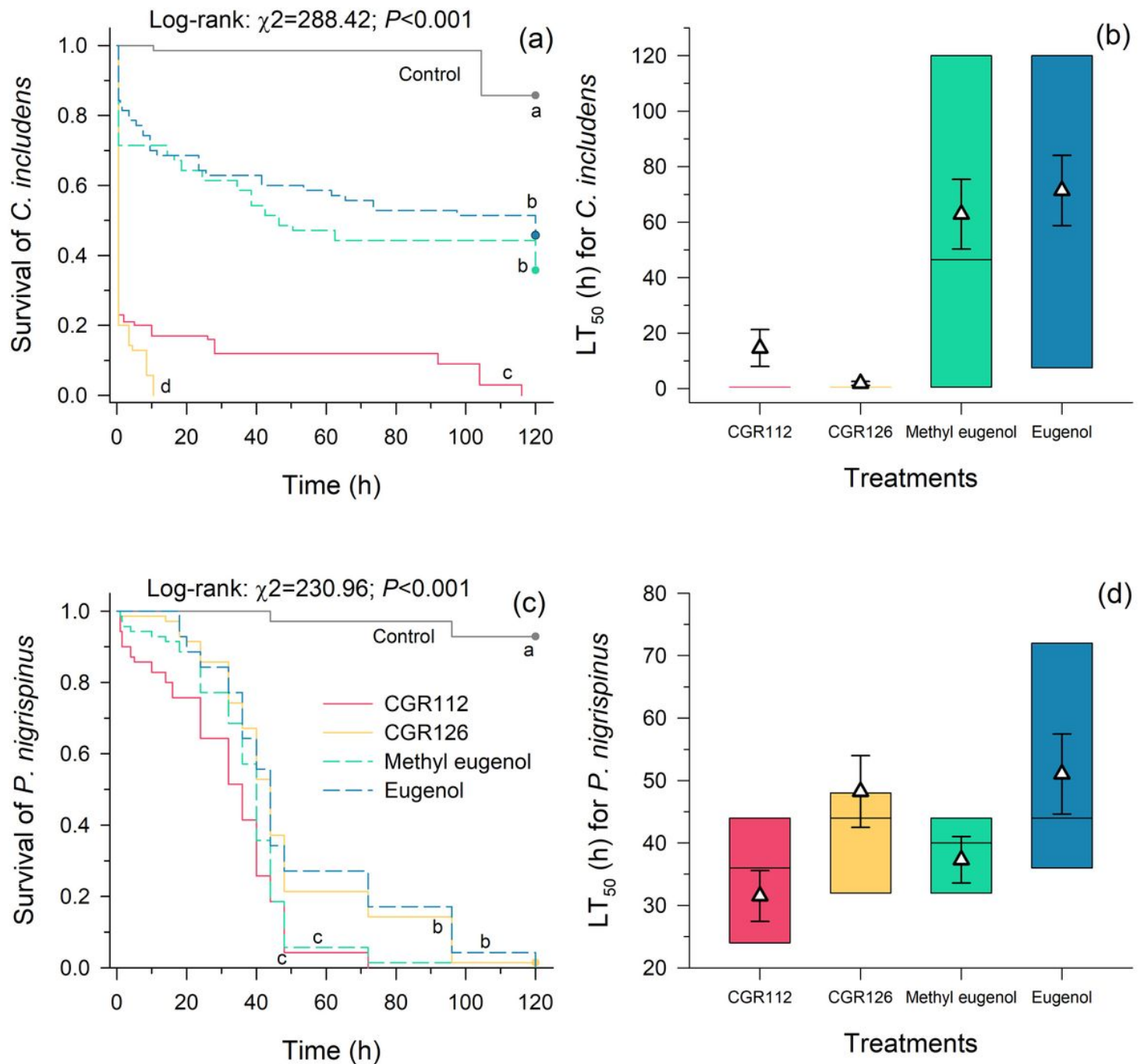


Figure 3

Survival and lethal time (LT₅₀) curves of 2nd instar larvae of *Chrysodeixis includens* (a, b) and 3rd instar nymphs of *Podisus nigrispinus* (c, d) exposed to LD₉₀s (see Fig. 1) of essential oils from accessions CGR112 and CGR126 from *Croton grewoides* and its major compounds methyl eugenol and eugenol. LT₅₀ = lethal time required to kill 50% of the population. Survival curves followed by the same letter do not differ significantly by the Holm-Sidak method. The ends of the boxes represent the 25th and 75th percentiles and the line inside the box represents the median. The white triangles represent the mean and the error lines the 95% confidence interval.

Figure 4

Duration of the egg stage; 1st, 2nd, 3rd, 4th and 5th instar nymphs; total nymphal stage and longevity of females and males of *Podisus nigrispinus* after exposure of 3rd instar nymphs to LD₃₀ of essential oils from accessions CGR112 and CGR126 of *Croton grewoides* and its major compounds methyl eugenol and eugenol. The error lines represent the 10th and 90th percentiles and the ends of the box the 25th and 75th percentiles. The solid line inside the box represents the median and the black circle the mean. Asterisked means differ from control by Dunn's non-parametric multiple comparison method at $P < 0.05$. Ct=control, 112=access CGR112, 126=access CGR126, MEg=methyl eugenol and Eg=eugenol.

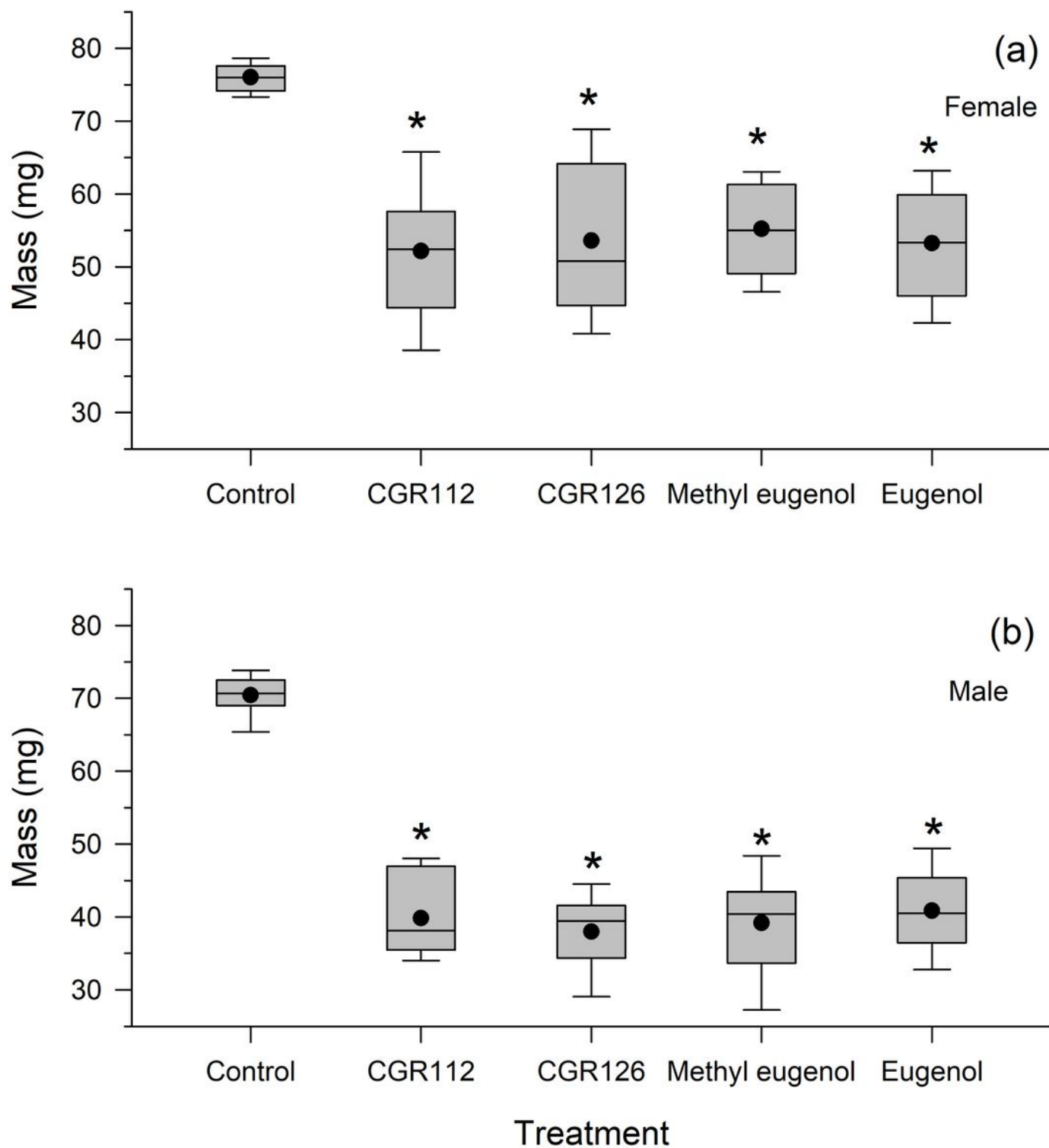


Figure 5

Mass of females (a) and males (b) of *Podisus nigrispinus* after 3rd instar nymphs exposure to LD₃₀ of essential oils from accessions CGR112 and CGR126 of *Croton grewoides* and its major compounds methyl eugenol and eugenol. The error lines represent the 10th and 90th percentiles and the ends of the box the 25th and 75th percentiles. The solid line inside the box represents the median and the black circle

the mean. Means followed by an asterisk differ from the control by Dunn's non-parametric multiple comparison method $P < 0.05$.

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