Journal of Experimental Agriculture International

Volume 44, Issue 12, Page 21-28, 2022; Article no.JEAI.93555
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Castor Seed Oil: A Promising Biomitecide for Sustainable Management of the Red Coffee Mite (*Oligonychus ilicis*) (McGregor, 1917) (Prostigmata: Tetranychidae)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AMH, TRD, RLA and PSFF designed the study and wrote the first draft. Author JRC performed the statistical analysis. Authors CMR, ABMP, AGA, MPG and IRP managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2022/v44i122074

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/93555

Original Research Article

Received: 06/09/2022
Accepted: 13/11/2022
Published: 19/11/2022

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ABSTRACT

The coffee red mite [*Oligonychus ilicis* (McGregor, 1917)] (Prostigmata: Tetranychidae) is one of the main causes of conilon coffee (*Coffea canephora* Pierre & Froehn) crop damage due to its phytophagous habit. Nowadays, environment and human harmful synthetic pesticides are used to control this pest. In this context, plant-derived bioactive compounds have been studied as a sustainable alternative for the pest mite management in crops. The aim of this study was to evaluate the castor (*Ricinus communis*) seed oil action on eggs, larvae, nymph (protonymph and deutonymph) and adults of *O. ilicis*. Coffee leaf discs (4 cm in diameter) containing 12 individuals of *O. ilicis* were sprayed with castor seed oil at concentrations of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% (v/v) using airbrush. The ovicidal and mortality activity of this extract were evaluated against *O. ilicis*. The effective concentration of castor oil to cause 50% inhibition of *O. ilicis* larvae hatching (LC 50) was 1.26% (v/v). *Oligonychus ilicis* treated with this oil at a concentration of 3.0% presented a larvae hatching percentage of 29.3%, lower than that obtained for the control treatment (79.1%). Castor seed oil 3.0% (v/v) was highly toxic to nymphs and adults of *O. ilicis* with mortality of 96 and 88%, respectively. Castor seed oil was effective for larvae hatching inhibition and nymphs and adults mortality of *O. ilicis*, being promising for the coffee red mite sustainable biocontrol.

Keywords: Coffea canephora; alternative control; mitecide; biopesticide.

1. INTRODUCTION

World coffee production exceeded 144.7 million bags in 2015, of which 41.5% were Conilon coffee produced in emerging countries, with Brazil being the second largest producer of this species in the world [1]. The coffee red mite, *Oligonychus ilicis* (McGredor, 1917) (Prostigmata: *Tetranychidae*), is one of the main phytophagous mites of the coffee tree (*Coffea* spp.), being one of the most important pests for the conilon coffee crop in Brazil [2]. Coffee tree red mite attack is favored by prolonged drought periods, affecting the entire crop if not controlled at the beginning of the infestation [3]. *Oligonychus ilicis* perforate the leaves cells and suck part of the cellular content to feed, causing a photosynthetic area of the leaves reduction, leaving them with a tanned appearance and covered with webs [2,4].

Chemicals belonging to the aldicarb, bifenthrin, chlorpyrifos, pyrethroids and organophosphate groups are used to reduce mite damage in crops [5,6,7]. However, the use of these chemicals leads to the selection of red mite populations resistant to these products, pest resurgence, secondary pest outbreaks, and compromised performance of natural enemies [8,9]. In addition, these pesticides are toxic to humans, being related to the development of diseases and effects such as tachycardia, hypertension, mydriasis, nausea, and vomiting occur, but more serious sequelae including respiratory failure, seizures and even death [10].

Alternative methods, such as the use of repellent biocompounds or insecticides/mitecides derived from plants, have been developed for the management of mites and insects pests [11]. *Jatropha curcas* L. oil at 1.0% (m/v) and leaf and stem extracts from this plant at 3.0% (m/v) caused mortality of *Tetranychus urticae* among 82.6 and 88.5% [12]. *Argemone mexicana* flower extract effectively acted on the mortality of *Culex quinquefasciatus* Say (Diptera: Culicidae) with LC 50 and LC 90 of 18.61 and 39.86 ppm after 24 hours, and 9.47 and 21.76 ppm after 48 hours, respectively [13].

Castor (*Ricinus communis*) has been described for its insecticidal/mitecidal capacity, presenting ricinine in its seed, a toxic alkaloid for mites and insects [14]. Castor seed oil was effective for controlling infestations of Mexican weevil *Zabrotes subfasciatus* (Boh.) and *Diaphania nitidalis* Cramer, 1782 (Lepidoptera: *Crambidae*) [15] (Mushobozy, Nganilevanu, Ruheza, & Swella, 2009). Although castor seed oil has been described as a promising biopesticide [15,14,16], there are no studies of this oil in the control of *O. ilicis*, the red coffee mite.

The objective of this work was to evaluate the mitecide effect of castor seed oil on the control of different stages of development of the coffee red mite.

2. MATERIALS AND METHODS

The experiment was carried out at the Instituto Federal de Educação, Ciência e Tecnologia do Espírito Santo – Campus Itapina (IFES – Campus Itapina), at the Laboratory of Agricultural Entomology and Acarology, in the first half of

2018. The experimental units were kept in acclimatized chambers at a temperature of 25 ± 1ºC, relative humidity 70 ± 10% and a 12-hour photophase.

2.1 *Oligonychus ilicis* cultivation

Oligonychus ilicis adults were collected in the field, in a conventional conilon (*Coffea canephora*) coffee plantation, located in the Santo Antônio do Mutum heritage site at Sítio Renascer, Colatina, Espírito Santo State, Brazil.

The adopted creation technique was adapted from Reis et al. [17]. The coffee leaves were collected from chemical-free crops located at the Instituto Federal do Espírito Santo, Campus Itapina and washed with sodium dichloroisocyanurate and distilled water. Afterward, they were dried and placed on cotton in a Petri dish (14.0 x 1.5 cm), with the leaves edges covered with moistened cotton to maintain the turgidity of the leaf and prevent the escape of mites.

After the procedure, the mites collected in the field were transferred to the plates and kept in acclimatized chambers (25±1ºC, 70±10% RH, and 12h photophase). Twenty adult females and 5 males of *O. ilicis* were removed from the breeding plates and transferred to Petri dishes as described above and kept for up to 24 hours to obtain eggs of the same age. This procedure was repeated for each development stage tested, with the same aim of age standardization. For each treatment, in each repetition, 12 individuals were left per plate.

2.2 Plant Extracts Production

Dry castor seeds were collected in an area free from chemical products at the Instituto Federal de Educação Ciência e Tecnologia do Espírito Santo (IFES) - Campus Itapina for vegetable oil production. In the laboratory, the castor seeds were peeled and then cold-pressed in a hydraulic press to extract the oil. For dilution, 3.0 mL of castor seed oil were added to 100 mL of distilled water and Tween® 80 adhesive spread (0.05%). Then, the mixture was stirred for 24 hours at room temperature on a magnetic stirrer. After this period, the material remained at rest for 20 minutes for decantation. After decantation, simple filtration was carried out in a cotton funnel to obtain a solid-free castor seed bean solution. The castor seed oil concentrations were 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% (v/v) as described above.

2.3 Bioassays

Castor seed oil was tested at concentrations of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% (v/v) on eggs, larvae, nymphs (protonymphs and deutonymphs), and adults obtained as described above. Eight replicates per treatment were performed, with 12 individuals per repetition. Each experimental unit consisted of a Petri dish (10.0 x 1.2 cm) lined with cotton inside. On top of the cotton, 4 cm diameter coffee leaf discs were added, with cotton moistened around them to maintain the turgidity of the leaf and prevent the escape of mites.

The spraying was carried out using an Alpha 2 model airbrush, connected to a calibrated compressor with a constant pressure of 1.3 psi and 2mL of castor seed oil solution, each formulated for each repetition. Distilled water and the adhesive spreader Tween® 80 (0.05% v/v) mixture was used as a control. The mitecide effect for eggs was evaluated from the 5th to the 7th day after application of the extract and for the other phases after 24, 48 and 72 hours after application.

The design used was completely randomized. Mortality data for each stage of development were corrected by Abbott's formula (1925) [18], and later submitted to variance and regression analysis tests by the SISVAR 5.6 program ($p \leq 0.05$). From the equations obtained, lethal concentration for killing 50% of the individuals or eggs (LC50) was calculated at each stage of development of *O. ilicis*. The differences in mortality in relation to time, for each concentration, in each stage, were submitted to analysis of variance and means were compared by the Tukey test ($p \leq 0.05$). The grouping of the toxicological effect was performed according to the model by Hassan et al. [19], when considering the percentage values of larvae hatching and corrected mortality, as follows: innocuous <25%; slightly toxic 25-50%; moderately toxic 51-75% and highly toxic >75%.

3. RESULTS AND DISCUSSION

3.1 Ovicidal Effect of Castor Seed Oil Against *O. ilicis* Eggs

The hatching percentage of *O. ilicis* eggs was inversely proportional to the concentration of castor seed oil (Fig. 1).

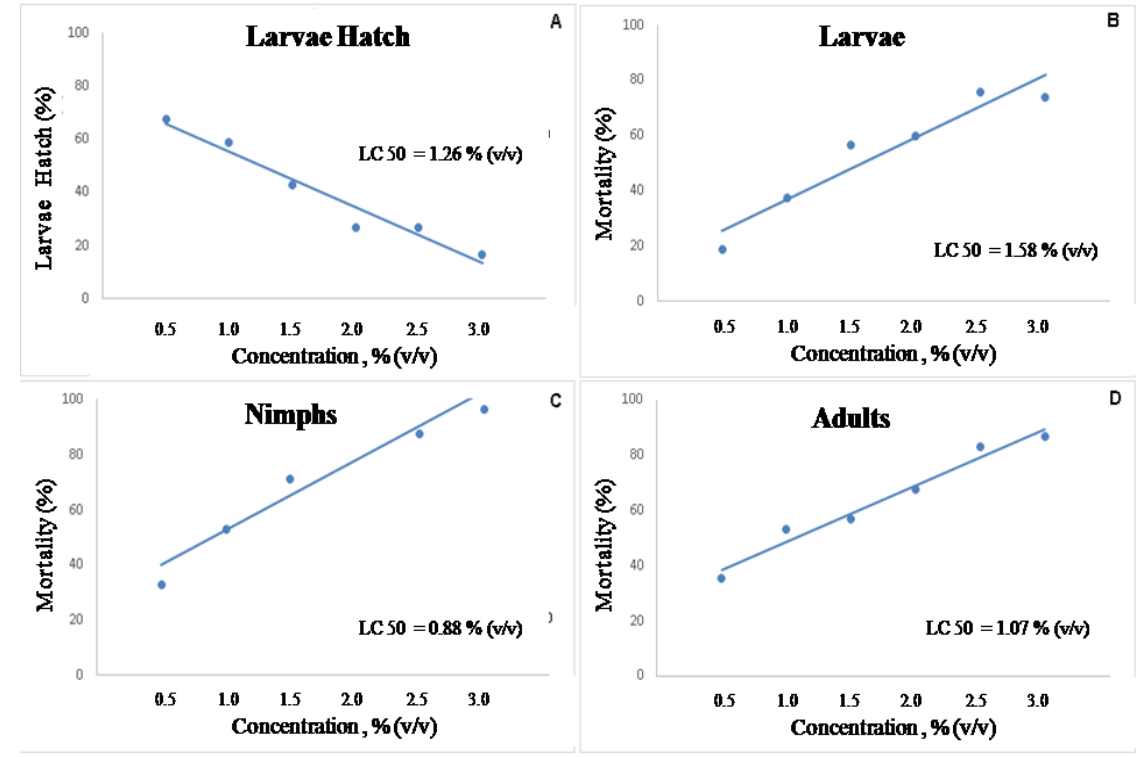

Fig. 1. Larvae hatch percentage (A), larvae (B), nymphs (C), and adults (D) mortality of *Oligonychusilicis* under different concentrations of castor seed oil (*Ricinus communis*), corrected by Abbott (1925) formula [18]

Treatments of *O. ilicis* eggs with castor seed oil at concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5 and 3% (v/v) showed hatching of 74.2, 67.6, 53.1, 41.7, and 29.3%, respectively, while the control treatment showed 79.1% hatching (Fig. 2).

The castor seed oil effective concentration to cause 50% of larvae hatching of *O. ilicis* (LC 50) was 1.26 % (v/v). These results agree with those of *Tetranychus urticae* eggs sprayed with Azamax® (insecticide/natural mitecide) showing viability inversely proportional to the concentrations of this product [20]. The increase in the Castor seed oil concentration inhibiting the hatching of larvae from *O. ilicis* eggs is justified by the presence of substances such as rhinoleic and oleic in this oil, described as toxic to oocytes of *Rhipicephalus sanguineus* (Latreille) and mosquito larvae, respectively [21].

Castor seed oil at 2.5 and 3.0% (v/v) was highly toxic to *O. ilicis* eggs, with corrected larvae hatching of 23.7 and 10.6%, respectively (Table 1).

The high toxicity of castor oil against *O. ilicis* eggs can be explained by its high concentration of ricinoleic acid, described as inhibiting the development of tick oocytes of the species *Rhipicephalus sanguineus* [22]. In addition, *Tetraniquid* eggs present a respiration mechanism through embryonic stigmas connected to the chorion and a specialized region of the intermediate membrane containing perforations [23]. These perforations are a likely way of penetration of bioactive compounds, justifying the high ovicidal activity of *R. communis* in eggs of this mite.

Mites parasitizing rabbits fed on feed containing castor oil have been reported to have oocytes with massive numbers of vacuoles, resulting from the toxic effects of ingested ricinoleic acid esters [22], agreeing with the high toxicity of this oil against *O. ilicis* eggs. Possibly the reduction in the number of larvae hatched *O. ilicis* with increasing concentrations of castor oil is related to the characteristics of embryonic development of this mite.

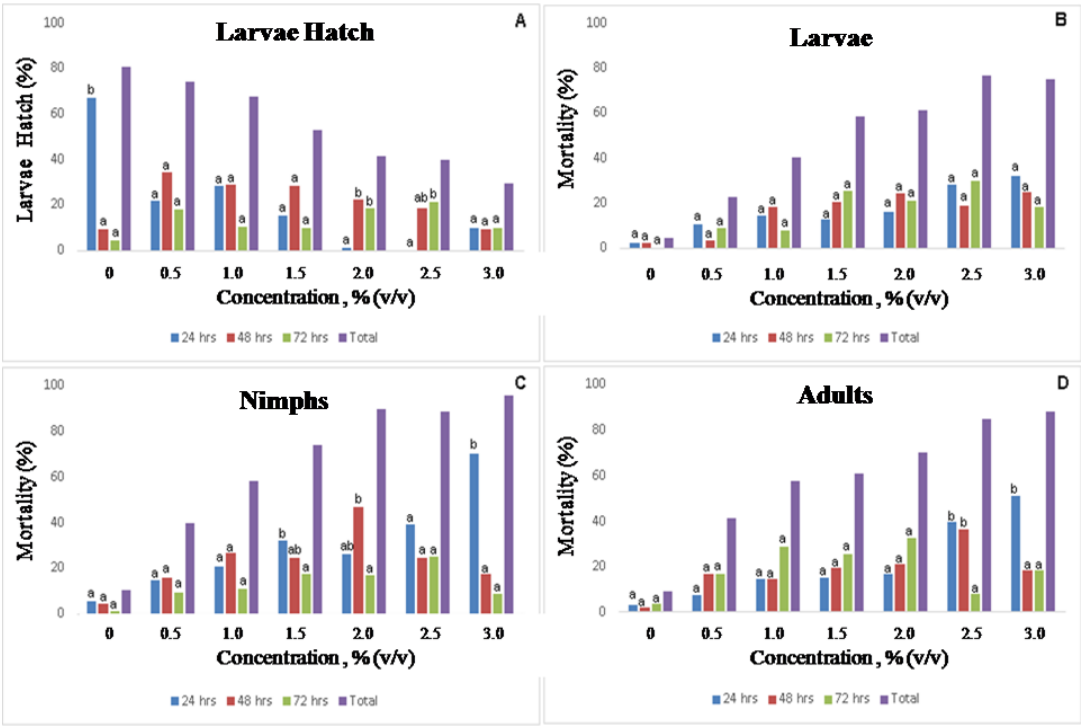

Fig. 2. Larvae hatch percentage (A), larvae (B), nymphs (C), and adults (D) mortality of *Oligonychus ilicis* at 24, 48, and 72 hours after the application of castor (*Ricinus communis*) seed oil

3.2 Larvae, Nymphs and Adults *O. ilicis* Mortality

The *O. ilicis* larvae, nymph and adult stages mortality was directly proportional to the castor seed oil concentration (Fig. 1), in agreement with the report of *Jatropha curcas* L. (*Euphrobiaceae*) extracts applied on the striped mite, *Tetranychus urticae* Koch (Acari: *Tetranychidae*), causing dose-dependent mortality [12]. The high toxicity of castor oil against *O. ilicis* can be explained by the high ricinoleic acid concentration in this oil, described as toxic to *Anopheles culicifacies* larvae, the vector of malaria [24].

Castor oil at 1.5, 2.0 and 3.0% (v/v) for nymphs, and 2.5 and 3.0%, for adults, caused higher *O. ilicis* mortality in the first 24 hours after application of the product (Fig. 2), agreeing with the report of high mortality of *Varroa destructor* in the first 24 hours of treatment with bifenthrin, fenpyroximate, fenazaquin, tebufenpyrad, chlorfenapyr and abamectin mitecides [25].

The effective castor oil concentrations to cause 50% mortality (LC 50) of larvae, nymphs and adults of *O. ilicis* were 1.58, 0.88 and 1.07% (v v^{-1}), respectively, lower than that observed for newly emerged female *Hypothenemus hampei* (3.49% v v^{-1}) [26], confirming the high lethality of this oil against *O. ilicis*.

Castor seed oil was highly toxic to larvae, nymphs and adults of *O. ilicis* at a concentration of 2.5% (v/v), for the first, 2.0, 2.5 and 3.0%, for the second, and 2.5 and 3.0%, for the third, respectively (Table). Higher *O. ilicis* mortality at higher castor oil concentrations is justified by greater cuticular penetration due to overload of the *O. ilicis* metabolic detoxification system [27]. The fact that castor oil was applied simultaneously on *O. ilicis* and its food (leaf disc) suggests that this mite was poisoned by three ways: ingestion, contact and respiratory tract, being the mode of action by contact faster than by ingestion [12,28,29].

Castor seed oil caused high mortality of eggs, nymphs and adults of *O. ilicis*, being a promising biocompound for the sustainable management of the red coffee mite.

Table 1. Castor oil toxicity at different stages of development of the coffee red mite (*Oligonychus ilicis*), with toxicological class of each concentration [19]

Development stage	Concentration	Larval hatch and mortality average (%)	Larval hatch mean and corrected mortality (%)	Toxicity
Larvae hatch	0	79.1	-	-
	0.5	74.2	67.4	Mild
	1.0	67.5	50.0	Mild
	1.5	53.1	40.8	Moderate
	2.0	41.7	26.3	Moderate
	2.5	39.6	23.7	High
	3.0	29.3	10.6	High
Larvae	0	4.6	-	-
	0.5	22.5	18.7	Innocuous
	1.0	40.4	37.6	Mild
	1.5	58.6	56.6	Moderate
	2.0	61.6	59.7	Moderate
	2.5	77.0	75.9	High
	3.0	75.2	74.0	Moderate
Nymphs	0	10.5	-	-
	0.5	39.6	32.5	Mild
	1.0	58.1	53.2	Moderate
	1.5	74.1	71.1	Moderate
	2.0	90.1	88.9	High
	2.5	89.4	87.8	High
	3.0	96.9	96.5	High
Adults	0	9.3	-	-
	0.5	41.6	35.6	Mild
	1.0	57.6	53.2	Moderate
	1.5	60.7	56.7	Moderate
	2.0	70.5	67.5	Moderate
	2.5	84.8	83.3	High
	3.0	88.1	86.9	High

4. CONCLUSIONS

The plant-based castor seed oil is ecologically correct and has a natural pesticide action. From this study, it can be concluded that castor seed oil is promising for the red coffee mite sustainable control due to its high efficiency against eggs, larvae, nymphs and adults of *O. ilicis* in laboratory assay.

Field tests applying castor seed oil for the *O. ilicis* control should be carried out in future works to confirm the efficiency of this oil for the red coffee mite management.

ACKNOWLEDGEMENT

We thank Fundação de Amparo à Pesquisa do Estado do Espírito Santo (FAPES) and Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico (CNPq) for the financial support and scholarships.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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