Effects caused by leaf extracts of castor *Ricinus communis* on the growth of larvae and development of pupae of *Culex quinquefasciatus*

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Introduction:

Castor *Ricinus communis* (Euphorbiaceae), a plant that has been distributed in tropical and warm areas of the world, has emerged as an alternative against pests of agricultural and veterinary medical home, including in this last area the mosquitoes. The biological activity of the extract of *R. communis* may be due to various compounds, including phenolics, terpenoids, flavonoids and alkaloids that may jointly or separately to help produce a larvicidal effect of inhibition of adult emergence and / or oviposition deterrent effect on species of *Anopheles arabiensis* and *Culex quinquefasciatus* (Elimam et al., 2009). However, still lacking many aspects to develop, so in this investigation was to evaluate the effect of the extracts with hexane, ethyl acetate and methanol leaf in castor early fourth instar larvae of house mosquito *C. quinquefasciatus* (Diptera: Culicidae).

Materials and methods:

- **Collection of plant materials.** Castor leaves (Fig. 1) were collected between December 2006 and March 2007 in the Colonial Gardens Casanueva the Municipality of Ecatepec de Morelos, Estado de Mexico, Mexico, between coordinates 19 ° 36 '56 " north latitude, 99 ° 03' 44" west longitude, at an altitude of 2251 meters over sea level.
- **Insect rearing (Fig. 2).** The fourth instar larvae of the mosquito *C. quinquefasciatus* were obtained from the insectary of the Interdisciplinary Research Center for Integral Development Regional (CIIDIR), the National Polytechnic Institute (IPN), Oaxaca, Oaxaca, México.
- **Preparation of plant extracts.** Castor leaves collected were dried in the shade at room temperature for 15 days, then crushed with a porcelain mortar, was added hexane and placed in boiling for four hours. Subsequently, the mixture was filtered, evaporated to the solvent and hexane extract was obtained. The previous extraction residue was added ethyl acetate or methanol and heated to a boiling point also about the same time, filtered and the solvent was removed under reduced pressure with a rotary evaporator and extracted with ethyl acetate extracts and methanol. Then be prepared eight milliliters of Polysorbate 20 or Tween 20 with distilled water to 10 parts per million, and this solution was added (by placing the bottle in a water bath) 100 parts per million of each extract, leaving the stock solution to 1250 parts per million. This initial concentration 4 ml was taken and mixed with 4 ml of distilled water to form the concentration of 625 parts per million, then this solution was measured 4 ml and were added to 4 ml of distilled water to prepare the concentration of 312.5 parts per million, and subsequent and so proceeded to make the concentrations of 156.2, 78, 39 and 26 parts per million.
- **Larvicidal bioassay (Fig. 3)**. The experiment was conducted in Oaxaca CIIDIR with the implementation of seven concentrations for each of three solvents castor leaf extracts with two controls getting a total of 23 treatments using a completely randomized design with a 3X7 factorial arrangement of treatments with four replications. Extracts were obtained with three solvents, hexane, ethyl acetate and methanol were applied to seven concentrations of each solvent (1250, 625, 312.5, 156.2, 78, 39 and 26 ppm) and two controls, one using tween and one without apply any substance.

The experimental unit consisted of a plastic cup capacity of 125 milliliters to 100 milliliters of distilled water and 20 fourth instar larvae of *C. quinquefasciatus*, which was added 1 milliliter of the desired concentration of the extract with an emulsifier.

Then, at the time that the Tween 20 control was 95% of pupae were recorded the duration and viability of larvae and pupae, larvae and pupae dead considering the normal movements that did not present to be disturbed with a needle dissection. This information is quantified growth inhibition index (IIC), as proceeded with Martínez *et al* (2009), using the formula of Zhang et al. (1993):

$$IC = \frac{\sum_{i=1}^{2} (\text{Number of live insects X Insect stage}) + \sum_{i=1}^{2} [\text{Number of dead insects X (Insect stage - 1)}]}{(\text{Total number of insect tested X Total insect stages})}$$

Where the phase of the insect was 1 and 2, corresponding to fourth instar larvae and pupae. The total number of insects tested was 80. The total phase of the insect was 2 (fourth instar larva and pupa).

With larval mortality data (when in the control + Tween 20 was formed ≥ 95% of pupae) was calculated median lethal concentration (LC50).

The data collection was continued with the surviving larvae and pupae, after recording the data to determine the IIC, recording duration and larval and pupal viability, being the larval period the number of days elapsed since the larva underwent treatment until completed its pupal stage and duration since the formation of the pupa to adult emergence. Larval and pupal viability was recorded as a percentage, considering the initial and final population in each phase.

- **Statistical analysis.** We performed an analysis of variance of the data using the JMP program version 7 (SAS, 2008) and mean separation was done with the Tukey test ($p \leq 0.05$). To stabilize variances, data from IIC and percentages of larval and pupal mortalities were transformed [sqrt (x)] before analysis. The mortality data of larvae were correlated with the values of concentrations of extract subjected to analysis with the procedure and Probit software (Raymond, 1985).

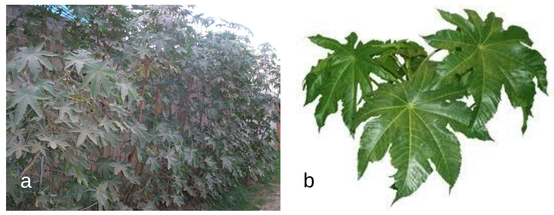


Fig. 1. ***Ricinus communis plants*** **(a),** ***Ricinus communis leaves*** **(b),**

Fig. 2. Breeding cages for *Culex quinquefasciatus* (a), population of larvae and pupae of *C. quinquefasciatus* in trays with water (b), fourth instar larvae of *C. quinquefasciatus* (c).

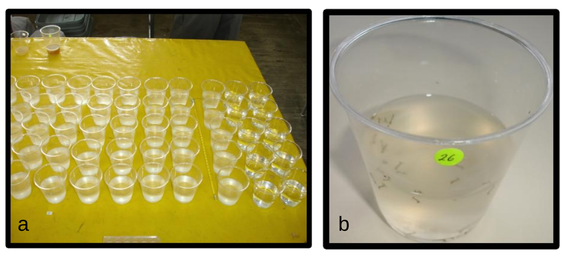


Fig. 3. Larvicidal bioassay (a), experimental unit (b),

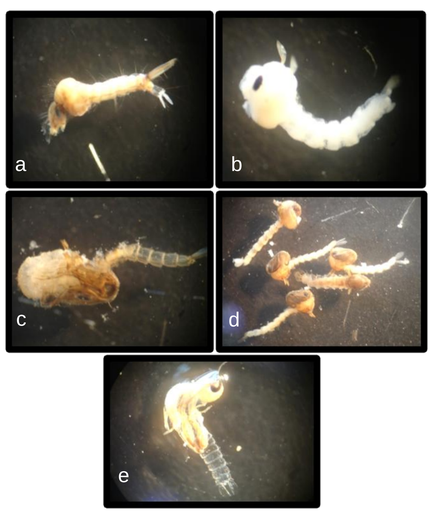


Fig. 4. Intermediates stages during metamorfophosis of a mosquito by the application of three leaf extracts of *R. communis* in fourth instar larvae of *C. quinquefasciatus*. Pupal abdomen retracted (a), unmelanized pupa with abdomen recurved (b), melanized pupa (c), pupa with adult form visible (d), adult partly escaped (e).

Results and Discussion:

• The IIC in 1250 and 625 parts per million of hexane extract are 0.34 and 0.44, the ethyl acetate extract are 0.34 and 0.46, and the methanol extract are 0.25 and 0.53, demonstrating moderate inhibition of growth of mosquito population are concentrations, so that 50% of individuals do not make it to adulthood with the hexane, ethyl acetate and methanol extract at 1250 ppm (Table 1 and Fig. 1). Compared to the latter solvent, Deng (2008) notes that ethanol, as ethanol extract of stem bark of castor *R. communis*, applied to third instar larvae of *C. pipiens pallens* extends from 0.5 to 2.5 days, the larval duration.

•The live larvae, which died with no treatment at the time of recording the IIC, larval growth followed by 3 and 5.5 days and pupal development of 5.7 and 7.3 days and had no significant difference with controls Tween and water (4.9 and 5.2; 6.9 and 6.9) respectively, while the extracts did not affect larval and pupal durations (Table 3).

•The highest mortality rates obtained with extracts of hexane, ethyl acetate and methanol in fourth instar larvae are 83.8, 100 and 88.8% at the concentration of 1250 parts per million, while the concentration of 156.2 parts per million activity of these extracts is very low with a range of 11.3 to 21.3% mortality (Table 1 and Fig. 2). These mortalities are correlated with the concentrations applied, provided the respective LC50 of 416, 417 and 761 parts per million.

•This toxicity is lower than that obtained in other investigations. Abduz (2009) indicated that the LC50 of the methanol extract of the leaf in fourth instar larvae of the mosquito *C. tritaeniorhynchus* is 93.94 parts per million and Batabyal et al. (2009) recorded LC50 91.62 and 62.2 parts per million at 24 and 48 hours after treatment with seed extract for third instar larvae of *C. quinquefasciatus*.

•The ethyl acetate extract of leaves are less toxic to larvae of *C. quinquefasciatus*, according to the comparison of data from this study with the results of Aouinty et al. (2006), who indicated that the ethyl acetate extract causes leaf 61.7 ± 3.8 and 77.5 ± 2.8% mortality in fourth instar larvae of *C. pipiens* at concentrations of 80 and 100 parts per million at 24 hours.

•This toxicity to mosquito larvae to ricinine is probably present in leaves (Kang et al., 1985; Upanasi et al., 2003) and substance reported at the ricinine is not soluble in hexane and low solubility in acetate ethyl in methanol (Ramos *et a*l., 2010).

•The three extracts at different concentrations, do not cause significant mortality in pupae, when applied to fourth instar larvae (Table 2).

•A concentration of 1250 parts per million three extracts dramatically inhibited larval growth, which is due to mortality of the population that the extension of its phase.

Table 1. Percentages of larval and pupal mortality and growth inhibition index (GII) of fourth instar larvae of *C. quinquefasciatus* treated with three leaf extracts of *R. communis*.

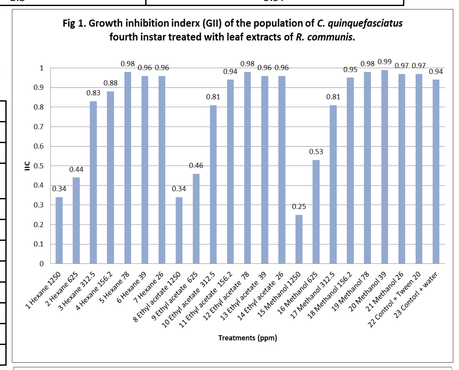
Concentration (ppm)	% Mortality (the control was formed in ≥ 95% of pupae)												IIC (the control was formed in ≥ 95% of pupae)					
	Larval						Pupal											
	Solvent						Solvent						Solvent					
	Hexane		Ethyl acetate		Methanol		Hexane		Ethyl acetate		Methanol		Hexane		Ethyl acetate		Methanol	
1250	83.8	a	100	a	88.8	a	16.3	a	0	c	0	c	0.34	c	0.34	e	0.25	c
625	87.5	a	98.8	a	25	bc	12.5	ab	0	c	0	c	0.44	cd	0.46	de	0.53	a
312.5	82.5	a	56.3	ab	25	bc	1.3	c	6.3	abc	0	c	0.83	c	0.81	b	0.81	a
156.2	15	cde	21.3	cd	11.3	cde	2.5	c	3.8	abc	0	c	0.88	a	0.94	a	0.95	a
78	5	def	20	cd	7.5	cdef	3.8	bc	1.3	c	1.3	c	0.98	a	0.98	a	0.98	a
39	0	f	0	f	0	f	0	c	1.3	c	1.3	c	0.96	a	0.96	a	0.99	a
26	1.3	ef	1.3	ef	1.3	ef	1.3	c	3.8	bc	1.3	c	0.96	a	0.96	a	0.97	a
Control + Tween 20	0						1.3						0.97					

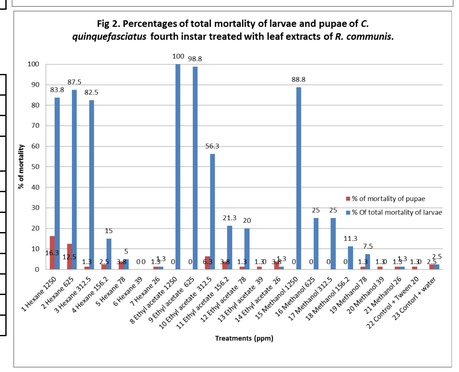
Table 2. Percentages of total larval and pupal mortality of fourth instar larvae of *C. quinquefasciatus* treated with three leaf extracts of *R. communis*.

Concentration (ppm)	% of total mortality											
	Larval (larva to pupa)						Pupal (pupa to adult)					
	Solvente						Solvente					
	Hexane		Ethyl acetate		Methanol		Hexane		Ethyl acetate		Methanol	
1250	83.8	a	100	a	88.8	a	16.3	a	-		5	abc
625	87.5	a	98.8	a	25	bc	12.5	ab	-		0	c
312.5	82.5	a	56.3	ab	25	bc	5	abc	13.4	ab	1.3	bc
156.2	15	cde	21.3	bcd	11.3	cdef	5	abc	11.3	abc	0	c
78	5	def	20	cd	7.5	cdef	7.5	abc	2.5	abc	1.3	bc
39	1.3	ef	0	f	0	f	0	c	1.3	bc	1.3	bc
26	1.3	ef	2.5	ef	1.3	ef	1.3	bc	3.8	abc	1.3	bc
Control + Tween 20	0						3.8					

Table 3. Larval and pupal duration of fourth instar larvae of *C. quinquefasciatus* treated with three leaf extracts of *R. communis*.

Concentration (ppm)	Duration (days)											
	Larval (larva to pupa)						Pupal (pupa to adult)					
	Solvent						Solvent					
	Hexane		Ethyl acetate		Methanol		Hexane		Ethyl acetate		Methanol	
1250	-		-		4	bc	-		-		6	a
625	-		3	c	5	abc	-		-		6.7	a
312.5	4.2	abc	3.9	abc	4.3	abc	-		5.7	a	6.3	a
156.2	4.4	abc	3.9	abc	4.4	abc	6	a	5.8	a	6.4	a
78	4.6	abc	4.5	abc	4.9	abc	6	a	6.3	a	6.6	a
39	5.4	ab	4.6	abc	5.3	abc	7.1	a	6.4	a	7	a
26	5.5	a	5.2	abc	5.3	abc	7.3	a	7	a	7.2	a
Control + Tween 20	5.2						6.9					





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