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Susceptibility of the blowfly, *Chrysomya putoria* (Diptera: Calliphoridae) to the ethanolic extracts of the medicinal plant *Moringa oleifera* (Magnoliopsida: Moringaceae)

Susceptibilidad de la mosca necrófaga *Chrysomya putoria* (Diptera: Calliphoridae) a los extractos etanólicos de la planta medicinal *Moringa oleifera* (Magnoliopsida: Moringaceae)

AGATHA BASTOS SANT'ANA DA SILVA TERTO[1] REBECCA LEAL CAETANO[1, 2] CÉSAR CARRIÇO[1, 3] MICHELE MARIA DOS SANTOS[4] JÚLIO VIANNA BARBOSA[1] JOSÉ AUGUSTO ALBUQUERQUE DOS SANTOS[2] ZENEIDA T. PINTO[1*]

[1] Instituto Oswaldo Cruz - IOC/ Laboratório de Educação em Ambiente e Saúde (LEAS)
[2] Instituto Oswaldo Cruz - IOC/Laboratório de Avalição e Promoção da saúde Ambiental (LAPSA)
[3] Instituto Brasileiro de Medicina de Reabilitação/IBMR/ Ânima
[4] Instituto Oswaldo Cruz - IOC/Laboratório de Biologia e Parasitologia de Maníferos Silvestres Reservatórios.

*** Corresponding author**
Zeneida T. Pinto. Laboratório de Educação Ambiental e em Saúde (LEAS), Pavilhão Lauro Travassos, Instituto Oswaldo Cruz, Fiocruz, Av. Brasil, 4365, Manguinhos - Rio de Janeiro - RJ - Brasil. zeneidateixeirapinto@gmail.com

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Abstract: Topical administration of *Moringa oleifera* leaf and seed ethanolic extracts concentrations (5, 10, 25, 50, 75, and 100 mg/L) were screened for insecticide activities against *Chrysomya putoria* (Diptera: Calliphoridae). Results showed that all tested leaf and seed extractconcentrations were toxic, delayed post-embryonic larval development, and reduced weight. As for *C. putoria* mortality, from newly hatched larvae to the adult period, all concentrations showed larvicidal power. However, above 20% of the mortality rate occurred in those treated with both leaf and seed extract concentrations. The ones treated with *M. oleifera* leaf extract concentrations presented the respective mortality rates: 50 mg/L (23 %), 75 mg/L (29 %), and 100 mg/L (30 %). While those treated with *M. oleifera* seed extract concentrations, the mortality rate observed was 25 mg/L (20 %), 50 mg/L (27 %), 75 mg/L (30 %), and 100 mg/L (32 %). The medicinal plant *M. oleifera* leaf and seed extracts proved to be a viable alternative for the population control of the medical veterinary and sanitary important blowfly *C. putoria*.

Keywords: Bioactivity, Diptera, insect vectors, medicinal plant, muscoid.

Resumen: La administración tópica de concentraciones de extractos etanólicos de hojas y semillas de *Moringa oleifera* (5, 10, 25, 50, 75 y 100 mg/L) se evaluó para actividades insecticidas contra *Chrysomya putoria* (Diptera: Calliphoridae). Los resultados mostraron que todas las concentraciones probadas de extractos de hojas y semillas fueron tóxicas, retrasaron el desarrollo larvario postembrionario y redujeron el peso. En cuanto a la mortalidad de *C. putoria*, desde larvas recién eclosionadas hasta el período adulto, todas las concentraciones mostraron poder larvicida. Sin embargo, más del 20 % de la tasa de mortalidad ocurrió en aquellos tratados con concentraciones de extractos de hojas y semillas. Los tratados con concentraciones de extractos de hojas de *M. oleifera* presentaron las respectivas tasas de mortalidad: 50 mg/L (23 %), 75 mg/L (29 %) y 100 mg/L (30 %). Mientras que los tratados con concentraciones de extracto de semilla de *M. oleifera*, la tasa de mortalidad observada fue de 25 mg/L (20 %), 50 mg/L (27 %), 75 mg/L (30 %) y 100 mg/L (32 %). Los extractos de hojas y semillas de la planta medicinal *M. oleifera* demostraron ser una alternativa viable para el control poblacional de la mosca *C. putoria* de importancia médico-veterinaria y sanitaria.

Palabras clave: bioactividad, Diptera, insectos vectores, muscoide, planta medicinal.

Introduction

Members of the family Calliphoridae (Diptera), known as blowflies, are vectors of several pathogens (Greenberg, 1973), and most of these flies have medical and forensic importance (Oliveira-Costa, 2011; Oliveira et al., 2007). The species *Chrysomya putoria* (Wiedemann, 1818) distribution is through the New World (Guimarães et al.,

1978). It is a cosmopolitan species and occurs in a variety of locations, invading residence areas, spoiling food, act as predators, parasites, pollinators, scavengers, and disease vectors (Gillott, 2005; Yeates & Wiegmann, 2005), besides, their larvae cause myiasis (Baumgartner, 1988; Prado & Guimarães, 1982).

According to Carriço et al. (2014), the use of synthetic chemical insecticides in pest control is dangerous because it affects man, and other animals and pollutes the environment. Pest management programs can induce insect resistance to the chemicals, and bioaccumulation, diminishing their effectiveness and becoming toxic to vertebrates (Prado, 2003; Scott et al., 2000; Valente et al., 2007).

Continuous use of non-selective substances can generate several problems such as affecting beneficial insects, leading to loss of insecticide effectiveness due to the natural selection of some generation of chemical resistant insects and contamination of the environment (Gerhardt et al., 2012; Marangoni et al., 2012).

According to Marangoni et al. (2012), for effective control with greater safety, selectivity, biodegradability, applicability, less environmental impact, and economic viability, new substances are necessary, such as the use of essential oils and plant extracts. This will make the most promising market for the consumption of biopesticides (Moreira et al., 2006).

Moringa oleifera Lam. (Magnoliopsida: Moringaceae) is among the plants whose extracts have insecticidal potential. This arboreal size plant originated in India, was then widely cultivated in the tropics all over the world, due to its rapid adaptation and development (Karadi et al., 2006). The high amount of vitamin C, proteins, phenolic compounds, tocopherols, and other nutritional properties present in the different parts of *M. oleifera*, such as seeds, fruits, and flowers, results in its wide use as human and animal food (Silva, 2017).

Phytochemical screening studies on *M. oleifera* ethanol leaf extracts identified four chemical constituents: tannins, flavonoids, coumarins, and alkaloids, and proteins (Saraiva et al., 2018).

More recent studies on ethanolic extracts of *M. oleifera* have demonstrated acaricidal activity on trombidiform mites (Heinz-Castro et al., 2021) and insecticidal activity on white flies, aphids, and beetles (Iqbal et al., 2023; Taaban, 2022).

Phytochemical screening studies on *M. oleifera* ethanol leaf extracts identified four chemical constituents: tannins, flavonoids, coumarins alkaloids, and proteins (Saraiva et al., 2018).

Lectins are proteins that bind to carbohydrates and have high biotechnological potential, including toxic activity on insects (Silva, 2017). The lectin´s insecticidal potential has been widely investigated against species of different insect orders such as Coleoptera, Diptera, Hemiptera, and Lepidoptera (Lam & Ng, 2011).

Katre et al. (2008) isolated two types of lectins from the seeds of *M. oleifera*. Similarly, *M. oleifera* seed extracts are an important ally against insecticide resistance, helping as a larvicide and pupicide against mosquitoes (Ashfaq & Ashfaq, 2012; Prabhu et al., 2011).

The insecticidal activity is usually evaluated through bioassays that incorporate the lectin intto artificial diets offered to insects, making it possible to investigate different parameters, among them, growth, development, fecundity, feeding inhibition, antimetabolic effects and mortality (Coêlho et al., 2009; Lawo & Romeis, 2008; Vasconcelos & Oliveira, 2004).

However, there is no report that considered the effect of *M. oleifera* leaf and seed extracts in the blowfly *C. putoria* life cycle. The purpose of this study is to evaluate the effect of *M. oleifera* seed and leaf ethanolic extracts in all *C. putoria* life cycle stages and its potential use in the integrated control of this species.

Materials and Methods

***Moringa oleifera* Extracts.** *M. oleifera* ethanolic leaf and seed extracts were prepared by the Laboratório de Avaliação e Promoção da Saúde Ambiental of Fundação Oswaldo Cruz – Rio de Janeiro, Brazil, according to the method of Yunes and Calixto (2001).

Insect Rearing. Wild *C. putoria* adults were captured on the campus of Fundação Oswaldo Cruz, Rio de Janeiro (latitude: 22°52′41″S, longitude: 43°14′41″W; 462 m a.s.l.), and were reared and maintained in the Laboratório de Educação em Ambiente e Saúde (LEAS), Fundação Oswaldo Cruz - Fiocruz, Rio de Janeiro, Brazil. Flies were maintained in plastic cages (30 cm x 30 cm x 30 cm) at room temperature (37 °C) with water and sugar powder. Ground beef for adult ovarioles maturation and oviposition stimulation was available. The bioassays were composed of larvae (L_1) of the third generation, reared under the following conditions: 27 °C ± 1 °C, 60 % ± 10 % RH, and an artificial photoperiod for 12 h.

Plant Extract. For extract concentrations, the *M. oleifera* crude extract was obtained from ethanol leaf and seed solvent dissolved in dimethylsulfoxide (pure DMSO – SIGMA, EUA). Then was tested in the following concentrations: 5 % (25 µL/ext + 475 µL/DMSO), 10 % (50µ L/ext + 450 µL/DMSO), 25 % (125 µL/ext + 375 µL/DMSO), 50 % (250 µL/ext + 250 µL/DMSO), 75 % (375 µL/ext + 125 µL/DMSO) and 100 % (pure extract), to obtain the six different test concentrations (5 mg/L, 10 mg/L, 25 mg/L, 50 mg/L, 75 mg/L and 100 mg/L).

Larvicidal Bioassays. The bioassays were performed in accordance with the protocol reported by Pinto et al. (2005a, b), from newly hatched larvae, transferred and grouped in a Petri dish, and then the six concentrations of *M. oleifera* ethanolic leaf and seed extracts were applied topically to the larvae bodies (L_1) by using an automatic pipette. Three replicates (n = 30) were used for each assay. The control groups consisted of groups of untreated flies and a group of treated flies with solvent only (pure DMSO). After inoculation, the larvae were kept in a recipient containing putrefied bovine meat (50 g), where they remained until they reached maturity (L_3) and then spontaneously left the diet. In sequence, they were moved to recipients with vermiculite, which were placed below the rearing containers, collected, individualized, had weight information recorded, and then transferred to glass test tubes containing vermiculite sealed with cotton plugs. After the emergence to adults, the insects were separated by gender and maintained in acclimatized chambers, programmed at 27 °C ± 1 °C, 70 % ± 10 % RH, and to an artificial photoperiod for 12 h.

Statistics Analyses. The biological activity of *M. oleifera* ethanol leaf and seed extract concentrations were observed for the *C. putoria* in each duration phase of development, sex ratio

calculation (nFemale/nFemale + nMale) (Rodrigues, 2004), morphological deformities analysis (if there were changes) and weight of mature larvae. The mortality rate and duration of each developmental period (larval, pupal, and newly hatched larvae to adult) were analyzed. The data were submitted to variance analyses (ANOVA 1; P < 005), followed by mean comparisons by Tukey test at a 5 % significance level (Zar, 1999). All statistical analyses were performed with the InStat program (version 3.05, 2000).

Results and Discussion

Topical administration of *M. oleifera* ethanol leaf and seed extracts on first instar larvae resulted in toxic effects in all tested concentrations, delaying post-embryonic development and reducing larval and weight, abnormal pupariation, and adultoid formation. The duration of *C. putoria* larval stage, pupal stage, and newly hatched adult stage treated with *M. oleifera* ethanol leaf and seed extracts showed similar and efficient results that are summarized in Table 1.

Regarding larval duration, in the use of *M. oleifera* leaf and seed extracts, both treatments revealed similarity between concentrations and significant delaying in the larval period at all concentrations, when compared to the pure control group and with pure DMSO (Table 1).

Other plant substances have shown the same life cycle reduction property of flies when exposed to these bioassays. This physiological parameter was also observed in tests with larvae (L3) of *Cochliomyia macellaria* (Fabricius, 1775) and *Lucilia cuprina* (Wiedemann, 1830) (Diptera: Calliphoridae) exposed to sub-lethal doses of essential oils (Chaaban et al., 2017, 2019a, b).

Oliveira et al. (2007) observed a total development period of 3.8 days of *C. putoria* larval instars (1st to 3rd instar), without substance, when reared in beef at the beginning of decomposition, maintained in acclimatized chambers, regulated at 27 °C ± 1 °C, 60 % ± 10% URA and 14-hour photophase. They followed the known development pattern of the most studied species of dipterans of the suborder Cyclorrhapha. Which instars development were a little higher than those presented in our study in the pure control groups (3.3 days) and in the DMSO group (3.2 days).

Musca domestica L., 1758 (Diptera: Muscidae) larvae (L3) and pupae fed with artificial diet added with *M. oleifera* ethanol extract of 10mg/L presented longer periods duration, 4.33 ± 0.57 and 5.00 ± 0.67 days, respectively (Kamel et al., 2019), partially diverging from the results found in the present study.

The essential *Cymbopogon citratus* (DC.) Stapf (Liliopsida: Poaceae) leaves oil has also been shown to increase the larval and pupal period of *M. domestica* (Pinto et al., 2015b). The causes of these changes in the larval period of insect development treated with plant metabolites are still unknown. However, some authors point out that some substances extracted from plants can affect the insect's endocrine system, acting on development or hormonal production (Cabral et al., 1999). According to Shalaby et al. (2016) and Chaaban et al. (2017), changes in larval cuticles, such as necrosis and reduced motility, as well as deformity in adults, are generally present when plant substances are used.

Moringa oleifera seed extracts showed greater impacts on the 2nd and 4th instar larval development stages of *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae), where 2nd-instar larvae were relatively more susceptible than 4th-instar larvae, with a high level of mortality documented in the study (Ashfaq & Ashfaq, 2012). In their study, Prabhu et al. (2011), when evaluating *M. oleifera* methanol seed extracts against *Anopheles stephensi* Liston, 1901 (Diptera: Culicidae), observed that there was larvicidal activity in larvae from the first to the fourth instar, with the first instar being the most susceptible than the others.

Several experiments using plant substances have shown their action on the development of various arthropods, increasing, or decreasing the larval and pupal period when exposed to these substances. Miyazawa et al. (1994), when testing the neolignan "licarin A" in *Drosophila melanogaster* (Meigen, 1830) (Diptera: Drosophilidae), observed that it directly interfered with its development. This interference was also present in a study carried out by Mukandiwa et al. (2012), who tested leaves extracts from *Clausena anisata* (Willd.) Hook. f. ex Benth. (Sapindales: Rutaceae), *Aloe zebrina* Baker (Liliopsida: Asphodelaceae), *Erythrina lysistemon* Hutch. (Fabales: Fabaceae) and *Spirostachys africana* Sond. (Malpighiales: Euphorbiaceae) to the second and third stages of the blowfly species *L. cuprina* and *Chrysomya marginalis* (Robineau-Desvoidy, 1830) (Diptera: Calliphoridae).

Regarding the pupal period stage, in the *M. oleifera* ethanol seed and leaf extract tests, only the 25 mg/L (4.2 ± 0.42 days and 4.2 ± 0.44 days) concentration, respectively, showed a reduction in this period, the other concentrations showed similar values in both tests, however, they showed an extension of the pupal cycle. Therefore, all concentrations presented change when compared with the pure control group 4.3 ± 0.48 days and with the control group with DMSO 4.3 ± 0.47 days (Table 1).

Although the method used and the species of dipteran are different, Prabhu et al. (2011), when testing *M. oleifera* methanolic seed extracts in *A. stephensi* (Diptera: Culicidae) larvae, found a pupae regression, in addition to the effective extract on all larval stages. This effect was also observed by Ohia (2014) that tested the *M. oleifera* aqueous seeds extract in *Anopheles gambiae* Giles, 1926 (Diptera: Culicidae), concluding that the extract was highly toxic to larvae, inhibiting the development of pupae.

According to Callander and James (2012) and Molento et al. (2020), the insects' response to toxic substances is related to their life cycles, the type test used, contact and/or ingestion, and the characteristics of each species of Diptera. Elkattan et al. (2011), by treating *M. domestica* with LC50 from *Acacia nilotica* (L.) Willd. Ex Delile (Magnoliopsida: Fabaceae), and Shaalan et al. (2005), by treating *A. aegypti* larvae with *Callitris glaucophylla* Joy Thomps. & L.A.S. Johnson (Equisetopsida: Cupressaceae) verified that these dipterans pupated quickly when there was an increase in toxicity. In a bioassay of pupicide fumigation and contact toxicity protocols using essential laurel, *Laurus nobilis* L. (Magnoliopsida: Lauraceae) oil against *M. domestica*, the higher doses induced an inhibition on pupae appearance and no development from pupae stage to adult stage in any of the doses tested as they performed the contact test of Chintalchere et al. (2020).

The duration period from neolarva stage to the adult stage in the treated *M. oleifera* ethanol seed and leaf extracts groups also showed similarity in concentrations, reducing the period at all concentrations. Otherwise, the leaf and seed extracts concentration of 10mg/L presented the shorter period being 6.9 ± 0.70 and 6.9 ± 0.74 days, respectively, when compared

to the pure control with 7.6 ± 0.78 days and to the DMSO control with 7.5 ± 0.70 days (Table 1). Tests with *Pouteria sapota* (Jacq.) H. E. Moore & Stearn (Ericales: Sapotaceae) crude leaf extract on *C. putoria* also shortened the larval period at concentrations 5mg/L – 4.0, 10mg/L – 4.1 and 25mg/L – 4.1, when compared to the control group (Carriço et al., 2014).

In another study on the malaria vector, *Anopheles gambiae* larvae, using *M. oleifera* aqueous seeds extracts, presented a delay in larval development (L3) for the pupal stage at lower concentrations (1160 and 1450 µg/ml) (Ohia et al., 2013). When analyzing the effects of the soluble *M. oleifera* lectin on *A. aegypti* larvae, they observed a delay in development and an increase in mortality (Ohia et al., 2013). Larvae (L4) treated with the WSMoL lectin, showed absence of underlying epithelium and increase in the intestinal lumen, appearing to be hypertrophied (Ohia et al., 2013). Interestingly, Coêlho et al. (2009) observed that lectins interfered on peritrophic matrix, altering digestive cells, interfering with development, and leading to *A. aegypti* larvae death. Carriço et al. (2015) also observed this delay in the *C. putoria* pupal period exposed to *P. sapota* leaves extract. These data corroborate the findings by Ohia (2014), when testing *M. oleifera* aqueous seeds extract on the malaria vector, *Anopheles gambiae*, that at the lowest concentrations (1160 and 1450 µg/ml) showed a delay in larval development (L3) at the pupal stage.

According to Oliveira et al. (2011), when *M. oleifera* lectins are introduced in insect feeding, they can interfere with larval development, weight, pupation, fecundity, and insect survival, but there are still few studies regarding the emergence of adults.

Additionally, data on larval weight (mg) and sex ratio were analyzed. Treated larvae with leaf and seed extracts were significantly affected by similar weight loss at all concentrations, being the lowest range of larval weight for the 100 mg/L concentration: with 27.30 mg minimum and 30.90 mg maximum for leaves extract and 27.10 mg minimum and 28.10 mg maximum for seeds extract.

According to Reis et al. (1994), larvae can reach the minimum weight for pupation, but it may not be enough to reach the necessary parameters for the accomplishment of the metamorphosis that originates in adults. Scavenger dipterans are more adapted to pupae even when the final weight is below the estimated average for other species (Hanski, 1987). Von Zuben (1998) drew attention to the need for minimum weight to flies for changing stage. In that study, he observed that the minimum weight required for *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) to become a pupa is 30.1 mg. In our studies, *C. putoria*, which belongs to the same family as the previous study, presented the lowest weights when treated with a 100 mg/L/Leaf/*M. oleifera* solution, remaining with 27.10 mg, and with 27.7 mg and 27.5 mg of weight in 75 and 100 mg/L/Seed/*M. oleifera* the solutions, respectively. This may explain the higher mortality at these concentrations, as they did not reach the minimum weight necessary to pupate.

When evaluating the effect of *C. citratus* (Liliopsida: Poaceae) and citral oil as an insecticide against flies, Pinto et al. (2015a) observed that the larval weight of *C. megacephala* was significantly affected. This parameter influences the vulnerability that the larva presents when reaching adulthood, becoming more fragile to environmental influences. This variation in weight was also observed by Carriço et al. (2014), when exposing *C. putoria* larvae to *P. sapota* leaf extract, they observed that at a concentration of 25 mg/L of this extract, the larvae became lighter (45.8 mg) and at concentrations of 5 and 10 mg/L they had more weight, such as: 47.0 and 51.2 mg, respectively. The same effect was observed in the lectins from *M. oleifera* leaves and seeds when introduced in an artificial diet. They exert their deleterious effects on the intestinal epithelium of insects, acting directly on the absorption of nutrients (Silva, 2017) and consequently interfering in the reduction of larval weight.

The *M. oleifera* leaf and seed extracts showed effective action in the embryonic stage of *C. putoria*, but no difference was observed between the different leaf and seed extracts action and the sex ratio (Table 2).

Mortality in the larval period of *C. putoria* was similar in both leaves and seed extracts, but the highest mortality rates observed were for 75 and 100 mg/L concentrations in both treatments. Regarding the control, there was no death in the pure control and DMSO control groups (Figure 1 A and B).

Kumar et al. (2013), when testing *C. citratus* oil on *M. domestica* larvae, found a high larvicidal and pupicidal activity with a LC50 of 0.41 µL/cm2 concentration, and an inhibition of 77.3 %. Singh and Kaur (2016) observed the same effect and reported a significant toxic effect of the *Azadirachta indica* A. Juss. (Magnoliopsida: Meliaceae) methanolic leaf extracts on *Chrysomya bezziana* Villeneuve, 1914 (Diptera: Calliphoridae) larvae, pointing to it as potential action against Diptera.

Table 1. Duration (mean days) period of *Chrysomya putoria* post-embryonic stages, for larvae treated with *Moringa oleifera* ethanolic seed and leaf extracts at different concentrations and for larvae from the pure and DMSO control groups.

Treatment (%)	Development stage					
	Larval stage		Pupal stage		Larvae to adult stage	
	Mean ± SD (days)		Mean ± SD (days)		Mean ± SD (days)	
	Leaf	Seed	Leaf	Seed	Leaf	Seed
Control	3.3 ± 0.46^{a}	3.3 ± 0.46^{a}	4.3 ± 0.48^{a}	4.3 ± 0.48^{ad}	7.6 ± 0.77^{a}	7.6 ± 0.77^{a}
Control DMSO	3.2 ± 0.43^{ac}	3.2 ± 0.43^{a}	4.3 ± 0.46^{a}	4.3 ± 0.46^{ad}	7.5 ± 0.70^{ac}	7.5 ± 0.70^{ac}
5mg/L	2.4 ± 0.50^{b}	2.3 ± 0.48^{b}	4.6 ± 0.46^{bc}	4.7 ± 0.45^{bc}	7.1 ± 0.32^{bd}	7.0 ± 0.29^{bd}
10mg/L	2.3 ± 0,49^{b}	2.4 ± 0.49^{b}	4.5 ± 0.50^{ac}	4.5 ± 0.50^{ac}	6.9 ± 0,70^{b}	6.9 ± 0.74^{b}
25mg/L	3.0 ± 0.11^{cd}	2.9 ± 0.19^{c}	4.2 ± 0.44^{a}	4.2 ± 0.41^{d}	7.2 ± 0.45^{bce}	7.2 ± 0.44^{be}
50mg/L	2.9 ± 0.64^{d}	2.9 ± 0.57^{c}	4.4 ± 0.67^{ac}	4.4 ± 0.63^{cd}	7.3 ± 0.62^{ade}	7.3 ± 0.69^{ade}
75mg/L	2.5 ± 0.50^{b}	2.5 ± 0.50^{b}	4.8 ± 0.31^{b}	4.8 ± 0.36^{b}	7.3 ± 0.54^{ade}	7.3 ± 0.56^{ce}
100mg/L	2.8 ± 0.576^{d}	2.9 ± 0.52^{c}	4.8 ± 0.31^{ac}	4.4 ± 0.50^{cd}	7.3 ± 0.76^{ade}	7.3 ± 0.81^{ade}

The letters $_{a, b, c, d}$ are used to demonstrate statistical differences calculated by ANOVA 1, ($P \leq 0.05$) followed by Tukey. HSD. X = Average and DP = Standard Deviation.

Table 2. Larval weight values and sex ratio of *Chrysomya putoria* treated with *Moringa oleifera* ethanolic seed and leaf extract at different concentrations, and for larvae from pure and DMSO control groups.

	Treatment (%)	Weight (mg)					
		Mean ± SD		IV		Sex ratio	
		Leaf	Seed	Leaf	Seed	Leaf	Seed
Chrysomya putoria	Control	40.5 ± 1.61[a]	40.5 ± 1.61[a]	30.00 – 43.80	30.00 – 43.80	0.50	0.50
	DMSO	40.5 ± 1.61[a]	40.5 ± 1.61[a]	30.00 – 43.80	30.00 – 43.80	0.50	0.50
	5 mg/L	39.5 ± 0.38[b]	37.2 ± 0.27[b]	39.00 – 40.20	36.80 – 37.40	0.49	0.51
	10 mg/L	38.8 ± 1.19[c]	37.2 ± 0.25[b]	37.40 – 40.20	36.80 – 37.40	0.51	0.49
	25 mg/L	39.0 ± 1.39[c]	33.4 ± 0.47[c]	37.10 – 40.60	32.60 – 34.20	0.50	0.49
	50 mg/L	37.1 ± 0.28[d]	30.3 ± 0.56[d]	36.50 – 37.40	29.70 – 30.90	0.49	0.51
	75 mg/L	30.3 ± 0.54[e]	27.7 ± 0.32[e]	29.70 – 30.90	27.30 – 28.10	0.50	0.50
	100 mg/L	29.5 ± 1.63[f]	27.5 ± 0.31[e]	27.30 – 30.90	27.10 – 28.10	0.50	0.51

#Values within a column followed by the same letter is not significantly different at the 5 % level according to Tukey's HSD

Regarding pupal mortality, the seed extract showed slightly higher mortality at concentrations 75 and 100 mg/L (10 % and 12 %), respectively, when compared to the *M. oleifera* leaf extract. No death in the control groups was observed (Figure 1 A and B). Pinto et al. (2015a, b) observed a high mortality, above 20 % at concentrations 5, 10 and 75 mg/L (21 %, 23 %, and 22 %), respectively, in *M. domestica* treated with *C. citratus* essential oil from Cuba. Regarding *C. putoria* development period from neolarva to adult stages, all concentrations showed larvicidal power. However, above 20 % of the mortality rate was observed in those treated with *M. oleifera* leaf extract concentrations as follows: 50 mg/L (23 %), 75 mg/L (29 %) and 100 mg /L (30 %). While to those treated with *M. oleifera* seed extract concentrations the results were 25 mg/L (20 %), 50 mg/L (27 %), 75 mg/L (30 %) and 100 mg/L (32 %). No death was observed in any of the control groups. The relationship between the concentration of natural substances and effectiveness may vary depending on the substance used, the type of target dipteran, and other environmental factors. In some situations, it is possible to observe phenomena known as hormetic effects, in which low concentrations of a substance can stimulate a beneficial effect, while higher concentrations can be harmful. This type of response is not exclusive to natural substances and can occur in different contexts (Calabrese & Baldwin, 2002).

Pinto et al. (2015a, b), when testing *C. citratus* oil from Cuba and Brazil on *M. domestica* larvae, found that the most sensitive period was from neolarva to adult stages, which reached a mortality rate above 60 % in both oils (Cuba/Brazil). Halim and Morsy (2005) related the same pattern with *Eucalyptus globulus* Labill. (Myrtales: Myrtaceae) oils at 0.1 %, 0.2 %, 0.3 %, and 0.5 % concentrations against 3rd instar larvae of *M. domestica*. The results showed 90 % of larvae mortality rate, pupae alterations, and fail to emerge in adults (Figures 2 and 3).

Abdel-Gawad (2018) in his study found that *M. oleifera* leaves extracts (Mo-LE) in low concentration showed larvicidal and pupicidal toxicity against the housefly (*M. domestica*). Pinto et al. (2015a, b), observed 80 % of mortality in *C. megacephala* treated with *C. citratus* essential oil and citral, where the values were below 50 % for LC50, but in *C. putoria*, a higher proportion of deformities in the adult stage, reaching above 85 % level, was observed.

Ohia and Ana (2017) analyzed the effectiveness of *M. oleifera* aqueous seed extracts as larvicide at different concentrations in *Anopheles gambiae*. They observed that in 24 hours, the larval mortality rate increased as the dose increased at different levels of treatment, and a lower mortality of 12.7 % was observed for the lowest concentration of 1160 µg/ml. These authors also observed that at high extracts concentrations, there was complete inhibition of pupation during exposure periods.

In a study with *M. oleifera* bark extract, Kamaraj et al. (2010) observed the action against larvae and adults of mosquito vectors of the filariasis *Culex gelidus* Theobald, 1901 and *C. quinquefasciatus* (Diptera: Culicidae). Mateus et al. (2017), tested ethanolic and aqueous extracts and oils from

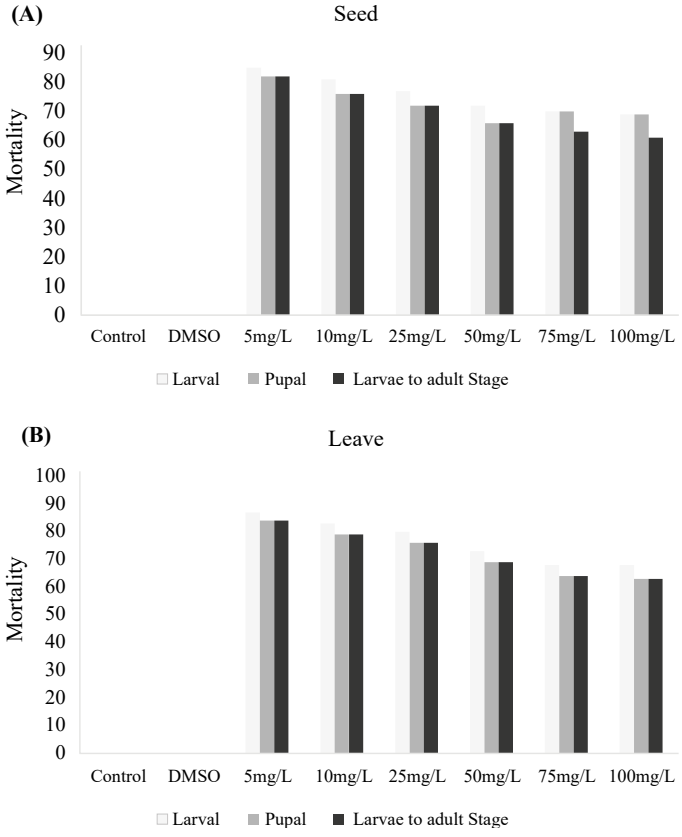

Figure 1. Mortality rate on larval, pupal and larvae to adult stage of *Chrysomya putoria* treated with *Moringa oleifera* ethanol seed (A) and leaf (B) extracts at different concentrations.

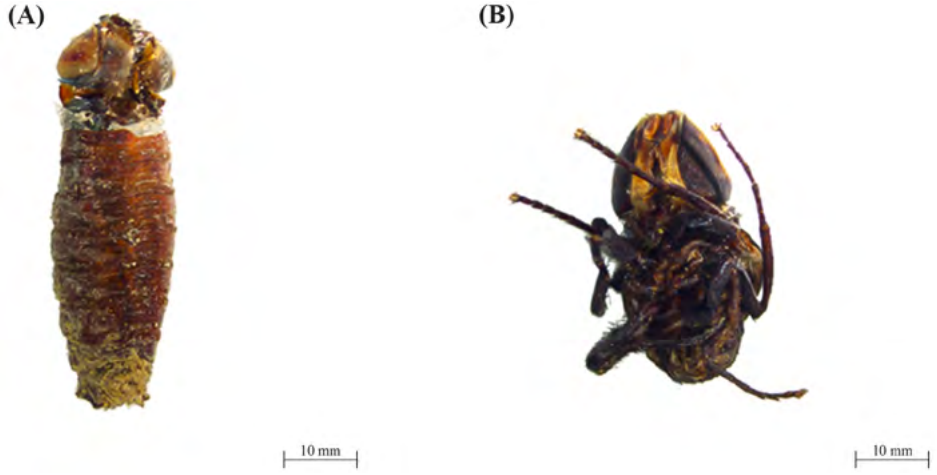

Figure 2. (A) *Chrysomya putoria* adult trapped in the pupal case during emergence attempt after treatment with *Moringa oleifera* seed extract. (B) *Chrysomya putoria* fly emerged with malformation after treatment with *M. oleifera* seed extract.

different *M. oleifera* structures (leaves, flowers, bark, seeds, and roots) on the weevil *Sitophilus zeamais* Motschulsky, 1885 (Coleoptera: Curculionidae), related that the oil caused 100 % of mortality at 10 ml/ L, while the ethanolic and aqueous extracts showed little efficacy at 50 ml/L.

Holtz et al. (2016) evaluated the toxicity of *M. oleifera* aqueous extracts from flowers, leaves and seeds on the spider mite *Tetranychus urticae* C. L. Koch, 1836 (Arachnida: Tetranychidae), observed a greater toxicity in the seed extract, with an estimated LC_{50} equal to 12.39 %. Rey (2010) also confirmed through scanning electron microscopy (SEM) that dipteran larvae exposed to plant substances presented histopathological and larvicidal effects.

Conclusions

The present study highlights the action potential of *M. oleifera* leaf and seed extracts on the post-embryonic development of the *C. putoria* blowfly, a vector of public health importance pathogens.

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Author Contribution

The first and the fourth author participated in the sampling and the writing of the final document.

The second and the third author participated in the sampling and taxonomic identification of Diptera, contributed to the analysis of the data and in the writing of the final document.

The fifth and the sixth author stated the objectives of the research, processed the plant material, contributed to the analysis of the data and to the writing of the final document.

The seventh author participated in the taxonomic identification of Diptera, contributed in the analysis of the data and in the writing of the final document.

Conflict of Interest

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