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Application of rotenone as a bio-insecticide for controlling the medically important blowfly, *Chrysomya albiceps* (Diptera: Calliphoridae)

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ABSTRACT

Chrysomya albiceps (C. albecips) is a native species of the tropical and subtropical African regions, is one of the most pervasive distributed Calliphorids, possessing detrimental ecological, medicinal, and veterinary implications. It is the causative agent of primary and secondary cutaneous myiasis and contributes to human and livestock infections as a mechanical carrier of several pathogens. The current research was to assess the effectiveness of rotenone as a plant toxin in controlling *C. albiceps* through evaluating its effect on different developmental stages. Rotenone has an impact on a variety of biological aspects in *C. albiceps*, as evidenced by decreased larval length and weight, the postponement of larval and adult development, and noticeably higher mortality rates. The effects of rotenone on various developmental parameters, egg development, egg load, oviposition capacity, and female preference were investigated. Ovarian examination of treated females revealed persistent retardation of follicular development, reducing egg load and inhibiting germarial activity. Rotenone additionally inhibits oviposition abilities and interferes with oviposition preference, decreasing the offspring's survival rate. Hence, rotenone could be considered a promising bio-insecticide to control *C. albiceps.*

Key words: Blowfly, *Chrysomya albiceps*, Diptera, Natural plant toxin, Rotenone.

1. Introduction

Chrysomya albiceps (Wiedemann, 1819) (Diptera: Calliphoridae) is one of the most important necrophagous, hemi-synanthropic species that favors elevated temperatures and humidity (Greenberg and Povolny, 1971;Aly et al., 2017). This species originally came from the old-World tropics, but it is widely distributed in several regions of the world (Zumpt, 1965; Guimarães et al., 1979; Mariluis 1980; Laurence 1981; Baumgartner and Greenberg 1984; Spradbery, 1991; Grassberger et al., 2003). It is regarded as a mechanical carrier for several pathogens that trigger diseases in domestic animals and people, as well as the causative agent of cutaneous myiasis (Zumpt, 1965; Grassberger et al., 2003; Mustika et al., 2016). According to the Public Health Significance of Urban Pests report by the World Health Organization, *C. albiceps* is considered an important disseminator of numerous food-borne infectious diseases (Bonnefoy et al., 2008). *C. albiceps* is regarded as a vector for infections including enterobacteria, enteroviruses, protozoan oocysts and cysts, helminth eggs, and fungas (Coessetin et al., 2021). Several studies are being conducted to control flies that cause myiasis in various parts of the world (Alahmed, 2004; Mohamed et al., 2016; Singh and Kaur, 2017). Conventional chemical insecticides are one of the most

extensively used methods of pest control. They are composed of numerous and varied chemical forms such as organophosphates, organochlorines, carbamates, and synthetic pyrethroids. The majority of which are neurotoxic and influence the nervous system, depending on their chemical structures, these chemicals interact with various sites (both target and non-target) (Gupta and Milatovic, 2014; Mdeni et al., 2022; Kumar et al., 2023). Moreover, the wide and prolonged use of these insecticides has resulted in accumulating their residues, thus leading to pesticide resistance. To mitigate the emergence of resistance, it is recommended to employ them alternately or in combination with other pesticides (Attia et al., 2013; Adhikari et al., 2022). Interest in plant extracts has increased considerably because of their relative efficiency against pests, their low mammalian toxicity, less persistence in the environment, and biodegradability, as well as their insignificant side-effects on non-target organisms (Isman, 2000, 2001, 2004; Tewary et al., 2005; Attia et al., 2012).

As we need to develop green, biodegradable, and eco-friendly products for a sustainable application (Bradu et al., 2022), the use of plant extracts appears to be an encouraging alternative strategy for pest control. Rotenone is a natural plant toxin isolated from various tropical and subtropical members of the family: Leguminsae that has attracted enormous attention as a pesticide. Rotenone is used commercially as a broad-spectrum insecticide in home gardens and plant crops, and as a veterinarian applicant in powdered formulation to control parasitic mites, lice, and ticks on different farm animals and pets (Gupta, 2007). The organ toxicity of rotenone has not been extensively studied because it is considered to be a specific insect neurotoxin that targets dopaminergic neurons (Kurpik et al., 2021). While the impact of rotenone on the ovaries has been investigated in some insects such as *Drosophila melanogaster* and *Musca domestica*, no data exists on its impact on *C. albiceps*. Therefore, the present study aims to fill this gap by determining the ability of rotenone to control *C. albiceps* through the evaluation of some developmental parameters: larval and adult growth and mortality rates, oogenesis, and oviposition preference of adult females.

2. Materials and methods

Rearing of *C. albiceps* in the laboratory

Insect larvae of *C. albiceps* were obtained from the stock colony maintained in the Entomology Laboratory at the Faculty of Science, Alexandria University. The larvae were housed in wooden cages (90 × 90 × 100 cm) and reared under laboratory conditions following a previously described protocol (Al-Shareef and Al-Qurashi, 2016). Laboratory colonies and all experimental flies were maintained under laboratory conditions at 29±3°C, 85±5% relative humidity (RH), and a photoperiod of 14 hrs of light:10 hrs of darkness.

Treatment of larvae flies with rotenone

Rotenone ≥ 95% was imported from Sigma-Aldrich (St Louis, MO, USA) in the form of white to yellow powder. According to Sudati et al. (2013), the (median lethal dose) LD_{50} of rotenone is about 0.35 mg/kg. To conduct the experiment, six male domestic rabbits, *Oryctolagus cuniculus*, weighing from 1400 g to 1700 g were divided randomly into two groups, control and treated, three rabbits each. Treated rabbits received a dose of rotenone equivalent to double the LD_{50} (about 1 mg per rabbit), adjusted for their specific weight (Haag, 1931). Sham-control rabbits received only 0.2 ml of ethanol (95%) for each one. All injections were made using BD U-100 insulin syringes (1 cc) through the ear vein (Aly et al., 2023). Thirty minutes after rotenone injection, treated rabbits died, and control ones were sacrificed mechanically by cervical disjoint.

All rabbits were then autopsied for liver removal, were then weighed and placed in plastic jars labeled control (C) and treated (T) in preparation for larval feeding of *C. albiceps*. One hundred one-hour-old larvae of *C. albiceps* were introduced into each plastic jar. The jars were kept at 28±0.1°C for rearing. Thirty larvae were randomly sampled from each control and treated jar at various time intervals 1, 6, 12, 18, 24, 30, 36, 42, 48, and 54 hrs and killed by immersing them in boiling water for 10 to 30s to prevent shrinkage (Adams and Hall, 2003) then their

lengths were measured under a dissecting microscope. Thirty larvae from each control and treated jar were weighed individually at 30, 36, 42, 48, and 54 hrs using a RADWAG scale (Model: WTC 200; Max 200 gm and d=0.001 gm). The larvae were subsequently removed from the livers, counted to figure out their mortality rate, and provided a diet of untreated beef liver to continue and complete their development. The duration of pupariation (start and end), as well as adult emergence time, were recorded.

Treatment of adults with rotenone

Twelve rearing cages were prepared for the control and rotenone-treated groups (six for each group). Each cage was provided with one hundred newly hatched flies (a sex ratio of 1:1) and was supplied with water and two types of food. The first type was a mixture of sugar, powdered milk, and yeast (1:1:1) that was introduced for 0-day-old adults. It was accessible all the time, until the end of the experiment, except for starvation hours. The second type was about 20 g of the beef liver that was added for the first time to one-day-old adults along with the first mixture, to promote the development of the gonotrophic cycle (liver serves as a protein source and oviposition site). The liver was only accessible to the adults for 12 hours per day for both groups. It was replaced daily with fresh liver for up to 7 days. Rotenone has minimal dissolution in water; hence it had to be dissolved in ethanol 99% first (Sudati et al., 2013) and then diluted by adding 10% sucrose solution (Navarro et al. 2014) to reach a final rotenone concentration of 250 µM, and a volume of 1% of ethanol (Sudati et al., 2013) of the total volume of the solution. The solution was applied to the treated group on a piece of cotton in a Petri dish. The rotenone solutions were fresh at the experimentation. Control group cages received 10% sucrose solution with a concentration of 1% ethanol served the same way. Both solutions were introduced to one-day-old adult flies that were starved for 1 hr. Flies from both control and treated groups were exposed to the corresponding solution for 10 hrs per day for a total of 4 days.

Mortality of adult flies

Adult mortality was evaluated by daily counting the number of dead flies till the end of the experiment (7 days). A total number of about 600 flies per control and treated group was included in the survival data, representing the sum of six independent experiments (100 flies each).

Ovarian examination of adult flies

Adult females of *C. albiceps* from both the Control and Treated groups were immobilized by chilling at -20 ºC for 5–10 min. The ovaries of 0- to 7-day-old females (10 females from each stage) were dissected in 0.85% saline solution under a dissecting microscope (Olympus, Japan) at x2 magnification to follow the stages of oogenesis. To examine the development of the ovary in both groups, the whole amount of the female reproductive system of 1- and 5-day-old females was photographed, and the diameter of each ovary was measured using ImageJ (Java-based image processing program) according to Litesy and Fine (2024). The ovaries were then placed on a glass slide with saline drops and the ovarioles were teased apart to be inspected. The ovarian follicles of both groups were examined by a compound microscope and photographed using a mounted digital camera (NikonC), and the lengths of the terminal ovarian follicles were measured using ImageJ. The number of follicles per ovariole was counted.

Female fecundity and oviposition

Beef liver, as one of the most attractive natural substances for adult blowflies (Bunchu et al., 2008), was used as an oviposition medium. First, twenty 5-day-old females from both the control and treated groups were provided with oviposition media within the cages and tested for their ability to oviposit. The number of females spotted laying eggs was recorded for both groups. Egg batching was observed on an hourly basis. At the end of 10 hrs, the total number of eggs was counted. Second, by the end of the 7th day (about 166 hrs) after eclosion, 10 females from both groups were placed separately within transparent jars with a slice of fresh beef liver to test their ability to lay eggs. The eggs were counted after 10 hrs.

Oviposition preference test and egg hatchability

The ability of female flies to choose a suitable site for their offspring was assessed by access to a food tube, or a smell tube that was placed 30 cm apart, at alternate angles, into 50×40×40 cm^3 labeled cages (Fig. 1). Pairs of 600 ml plastic bottles with the lid cut out were prepared as food tube and smell tube for each cage. Liver slices were placed in the food tube as a suitable resource for the hatching larvae to feed and for proper humidity for the eggs. On the other hand, the smell tube had no food for the larvae, but only strong repellent odors mixed with liver odor, and it was significantly less humid. Twenty random gravid females (control or treated) were placed into each cage. Fresh pieces of beef liver were left to rot for a few hours, to emit a strong attractive smell for gravid females. The bottles' opening was covered with a cling film that was pierced with large holes to allow flies to enter. No actual food was introduced to the smell tube, but a piece of cotton soaked in liver seep and a few drops of essential oils that are known to repel flies (mint, lavender, and citrus oil) (Renkema et al., 2016), to allow the odors to spread preventing humidity, a dark fabric with three plies was laid above the soaked cotton. All the flies in the experiment were 5-day-old nulliparous females. After 1 hr of starvation, the food tube and the smell tube bottles were introduced inside the control and rotenone-treated cages. The number of flies inside each tube was counted on an hourly basis, and oviposition times and places were recorded. After the experiment time was over (10 hrs), the total number of eggs laid was counted, and these eggs were allowed to hatch in the same bottles. The eggs that failed to hatch (dry eggs) were also counted.

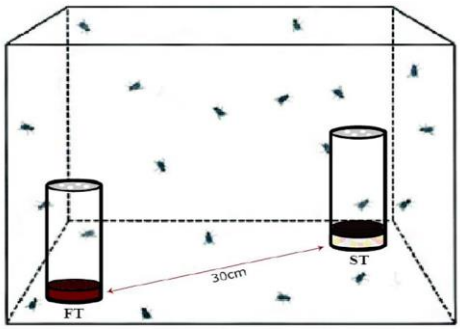

Fig. 1. Experimental design for assessing oviposition site preference in female flies. The configuration consists of a food tube (FT) and a smell tube (ST). Female flies were permitted to select freely between the two tubes, replicating natural conditions for site selection.

Statistical analysis

Data were subjected to analysis using the IBM SPSS software package, version 20.0 (Armonk, NY: IBM Corp). The normality of the distribution was checked utilizing the Kolmogorov-Smirnov test. The acquired results were judged at $p< 0.05$ and $p< 0.001$ for significant and highly significant differences, respectively, using both the student t-test and Mann-Whitney test to compare the two studied groups.

3. Results

Effect of rotenone on larval development, mortality and adult emergence of *C. albiceps*

After the first few hours of larval exposure to rotenone, no significant variations in length were observed between the control and treated larvae. Nevertheless, after 6th hour of the experiment, the treated larvae exhibited a comparatively slower rate of development than control ones (Table 1). Significant decreases ($p\leq 0.001$) in average body lengths and weights of treated larvae compared to controls were recorded during the developmental period up to 54 hrs (Table 1 and 2). The average mortality percentage of larvae, pupae, and adults of the control and rotenone-treated *C. albiceps* was shown (Fig. 2). Compared to the control group, the mortality of larvae and pupae of the insects treated with rotenone was significantly higher ($p\leq 0.05$). The results indicated a substantial difference ($p< 0.05$) in the pupariation time, both at the initiation and end times, between the larvae of the control group and those treated with rotenone. The pupariation phase of treated larvae exhibited a delay of 5 hours in initiation and a 16-hour delay in completion, as compared to the control group. Correspondingly, rotenone-treated insects showed a significant increase in adult emergence duration compared to controls, both at the initiation and end times (Fig. 3).

Table 1. Mean body length of larvae *C. albiceps* versus time of exposure

Time (hrs)	Larval lengths mm (Mean ± SD)	
	C (n =30)	T (n =30)
1	1.88 ± 0.18	1.94 ± 0.13
6	2.36 ± 0.22	2.23 ± 0.35
12	2.99 ± 0.18	2.70 ± 0.38*
18	4.27 ± 0.24	3.82 ± 0.63*
24	5.80 ± 0.23	4.95 ± 0.80*
30	6.80 ± 0.31	5.49 ± 0.84*
36	7.22 ± 0.29	6.31 ± 0.61*
42	8.09 ± 0.26	7.39 ± 0.55*
48	9.98 ± 0.57	8.45 ± 0.51*
54	11.24 ± 0.32	10.08 ± 0.76*

C: Control; T: Rotenone-treated; *: statistically significant at $p \leq 0.001$ using Student t-test.

Table.2. Mean body weights of larvae C. *albiceps* versus time of exposure.

Time (hrs)	Larval weights mg (Mean ± SD)	
	C (n =30)	T (n =30)
30	4.03 ± 0.72	2.23* ± 1.14
36	8.67 ± 0.96	4.90* ± 0.88
42	12.80 ± 1.10	7.10* ± 1.54
48	21.73 ± 2.35	12.19* ± 3.17
54	32.97 ± 2.65	20.97* ± 2.93

C: Control; T: Rotenone-treated; *: statistically significant at $p \leq 0.001$ using Student t-test.

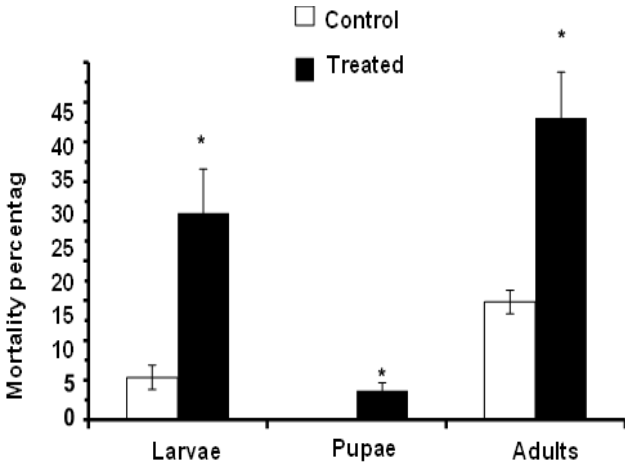

Fig. 2. Mean mortality percentage (± SD) of larvae, pupae, and adults of the control and rotenone-treated *C. albiceps*. *****: statistically significant at $p \leq 0.05$ using Student t-test.

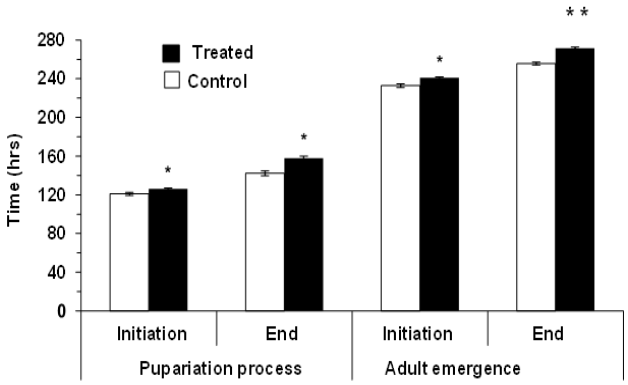

Fig. 3. Phylogenetic mean pupariation, and adult emergence time (hrs) (initiation and end times) (±SD) of the control and rotenone-treated *C. albiceps*. *: statistically significant at $p \leq 0.05$ using Student t-test. **: statistically significant at $p \leq 0.001$ using Student t-test.

Rotenone's impacts on female *C. albiceps* oogenesis

The follicles within the *C. albiceps* ovaries show a synchronous continuous development that follows a series of definite cytological events, which can be categorized into eight distinct stages. The ovarian developmental stage, female chronological age, the lengths of the developing follicles at each stage of oogenesis, as well as the egg load of both the control and rotenone-treated females are estimated (Table 3). The results demonstrate that rotenone treatment affected female chronological age at each developmental stage of oogenesis, and prolonged duration of development compared to the controls. The follicle's structure was impacted by the administration of rotenone, causing retardation in its development accompanied by notable reductions ($p \leq 0.05$) in follicle length at each stage of oogenesis, when compared to the normal development of follicles. Egg load, which was recorded only at the final ovarian stage (VIII), exhibited a substantial decrease ($p \leq 0.05$) for treated females to controls.

Table 3 Female chronological age, length of the developing follicle, and egg load of females *C. albiceps* at each developmental stage, for I, II, III, and IV

Ovarian stages		Time (hrs)		Length (mm)		Egg load (Mean±SD)	
		C	T	C	T	C	T
Previtellogenesis	I	0–12	-	0.07–0.08	-	0	-
	II	10–40	10–72	0.11–0.16	0.11–0.14*	0	0
Vitellogenesis	III	28–68	48–76	0.21–0.28	0.16–0.21*	0	0
	IV	56–72	56–95	0.33–0.51	0.29–0.50*	0	0
	V	60–76	60–120	0.72	0.66*	0	0
	VI	68–80	68–>120	0.941	0.93*	0	0
	VII	76–90	80–>144	1.340	1.21*	0	0
Gravid	VIII	80-95	95–>166	1.410	1.22*	260.6±2.70	153.0±128.6**
						n=20	n=20

C: Control; T: rotenone-treated; (Mean±SD), the lengths represent the range of early and late stages

Stages of oogenesis of adult control and rotenone-treated female flies

before treatment) (Fig. 4a and b). At the end of stage II (control 10–40 hrs) the control insects' follicles undergo a development process, resulting in a slightly ellipsoidal shape, within the nurse chamber, residing most of the follicle, the nurse cells are more scattered. Few yolk granules can be observed surrounding the oocyte, the second follicle starts to develop (Fig. 4c). At late stage II of treated insects (treated 10–72 hrs), the first follicle is much reduced in size compared to the controls, while the second follicle is not developed (Fig. 3d). In stage III (control 28–68 hrs), the 1st follicle is enlarged and the yolk acquiring a basal dome shape, while the 2nd follicle is completely separated (Fig. 5a). At late stage III of treated insects (treated, 48–76 hrs), the 2nd follicle is distinct and not separated from the germarium, the yolk granules are less accumulated than those of the controls (Fig. 5b). The 2nd follicle in treated insects is clearly separated in stage IV (treated, 56–95 hrs), while it was clearly noted that the oocytes of the control group (56–72 hrs) occupied a greater portion of follicle, approximately 40%, compared to the treated group (Fig. 5d and c, respectively).

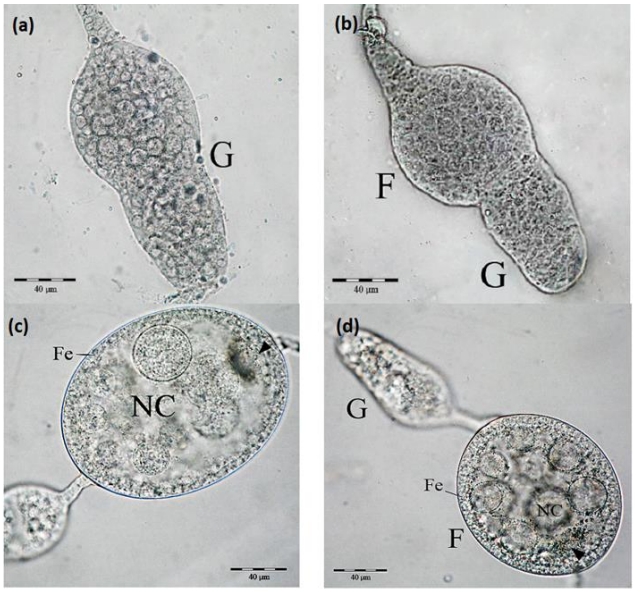

Fig. 4 Previtellogenesis stages of female *C. albiceps*, stage I of oogenesis of control insect **(a)** and rotenone-treated female **(b);** stage II of control insect **(c)** and rotenone-treated insect **(d).** Germarium (G) starts developing the first ovarian follicle (F), (NC) nurse cells, (Fe) follicular epithelium, and (arrow heads in **c** and **d**) yolk granules.

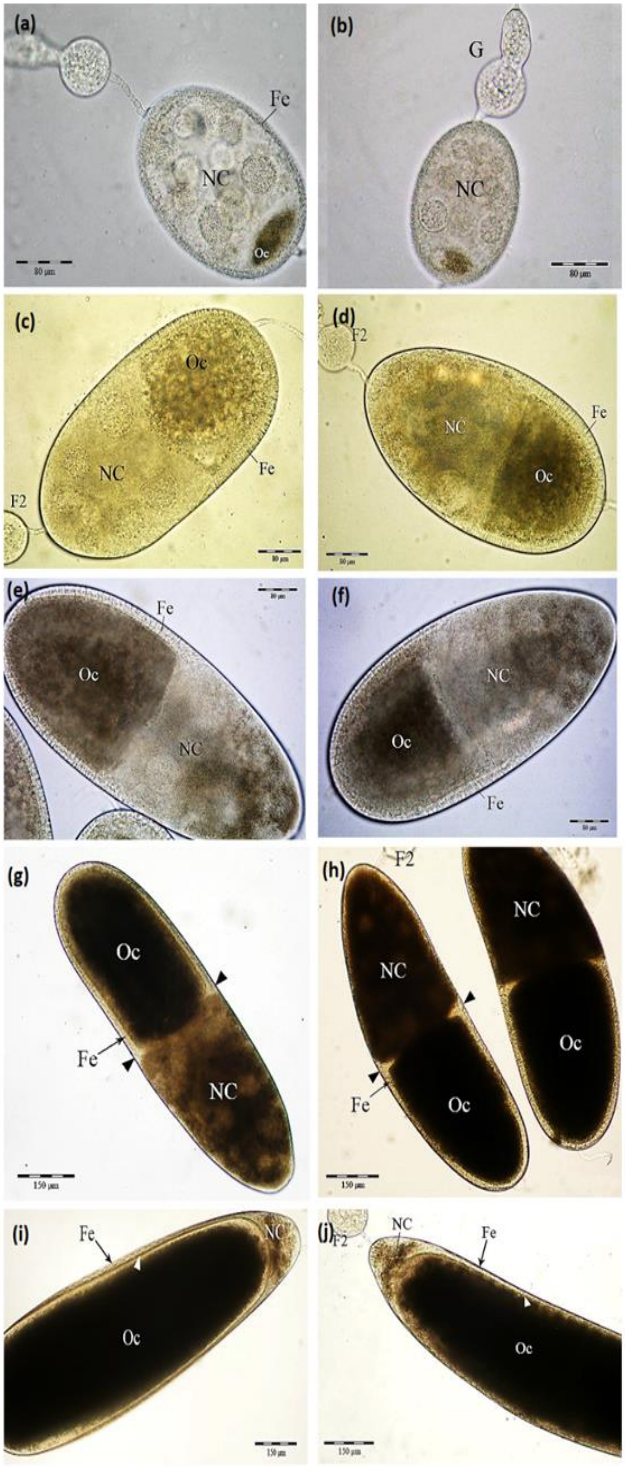

Fig. 5 Vitellogenesis stage of female *C. albiceps***.** stage III of control insect **(a)**, stage III of rotenone-treated insect **(b)**, stage IV of control insect **(c)**, stage IV of rotenone-treated insect **(d)**, stage V of control insect **(e)**, stage V of rotenone-treated insect **(f)**, stage VI of control insect **(g)** stage VI of rotenone-treated insect **(h)**, stage VII of control insect **(i),** stage VII of rotenone-treated insect **(j).** (NC) nurse cells; (F2) second follicle; (G) germarium; (F) follicle; (Fe) follicular epithelium; (Oc) oocyte; (black arrow heads in **g** and **h**) a barrier between NC and Oc; (white arrow heads in **i** and **j**) a vitelline membrane.

The follicle lengthens significantly at stage V of control insects (60–76 hrs, reaching 0.72 mm (Table 3), with the oocyte filling roughly half of the follicle (Fig. 5e). Comparatively, in treated insects stage V (60–120 hrs) the follicle exhibited a significant reduction in length (0.66 mm) (Table 3), with the oocyte occupying approximately 40% of the follicle (Fig. 5f). Stage VI of the control (68–80 hrs) and treated insects (68 >120 hrs) (as depicted in Fig. 5 g and h, respectively) showing similarities, particularly in the elongation of the follicle. This process results in a constriction, exhibiting a slight barrier between the oocyte and the nurse chamber, where the oocyte comprises approximately 50% of the overall length. Treated insects' follicular length is relatively shorter, with a significant difference compared to control insects (Table 3). The nurse cells at the proximal tip of the follicle degenerate within Stage VII (control, 76–90 hrs; treated, 80–144 hrs), while the follicular cells start to secrete the vitelline membrane, which is less defined in treated insects than controls (Fig. 5 i and j, respectively). The follicular length was 1.34 and 1.21 mm for the control and treated insects, respectively (Table 3).

Both control and rotenone-treated insects develop a mature gravid egg at the end of vitellogenesis, with a longer duration in treated females (95 >166 hrs) compared to the control ones (80–95 hrs). The development of the third follicle started to separate from the germarium of a normal mature egg (Fig. 6a); however, this was hindered in rotenone-treated insects. (Fig. 6b). Conclusively, treatment with rotenone incited an inhibition of germarium activity, as revealed by a delayed growth of the second follicle in treated females (56 hrs) compared to the control group (40 hrs). Accordingly, the third follicle in treated females was not noticed within a time frame of 166 hours, though it was developed within 80 to 90 hrs in control ones. Egg maturation was accomplished 95 hours after emergence in control insects, while it was postponed up to 166 hours with treated groups. It is worth noting that the development was halted in some treated females after the fifth stage.

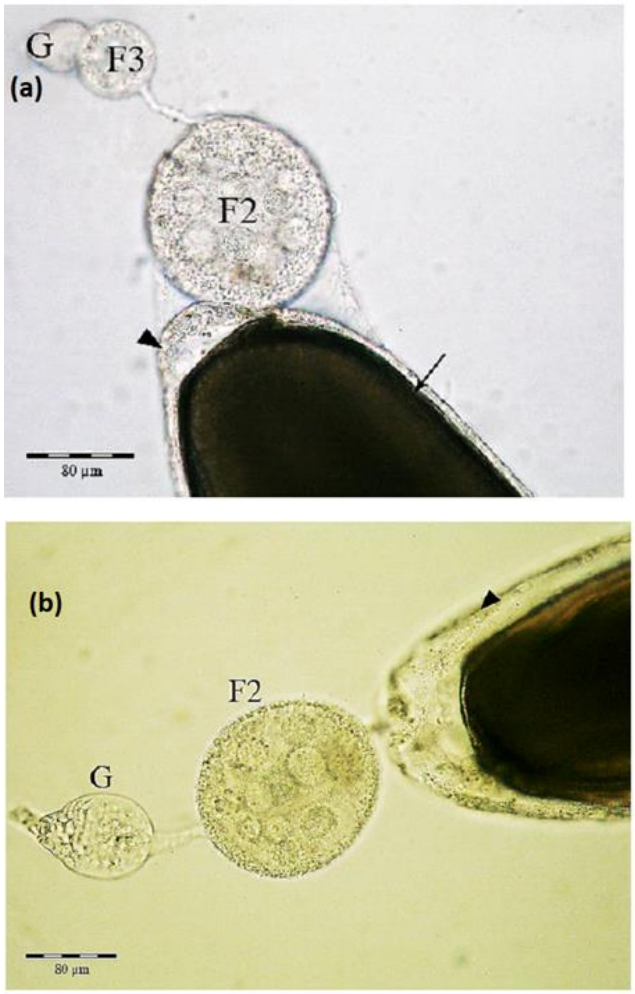

Fig. 6. Gravid stages (VIII of oogenesis) of female *C. albiceps*. stage VIII of control insect **(a)**, stage VIII of rotenone-treated insect **(b)**. (F2) second follicle; (arrow heads in a and b) a vitelline membrane; (G) germarium; (arrowhead in **a** and **b**) residues of follicular epithelium; (F3) third follicle.

Effect of rotenone on oviposition and fecundity of adult females *C. albiceps*

The administration of rotenone substantially increased the percentage of 5-day-old female flies that failed to oviposit during the experiment's timeframe, as illustrated in Fig. 7. The oviposition failure rate among the treated females was about 80%, whereas it was 22.72% in the control group. On the 7th day after emergence, 30% of the treated females displayed oviposition failure, whereas no such failures were detected in the control group. The fecundity of 5-day-old females treated with rotenone showed a highly significant reduction ($p \leq 0.001$), with an 81.29% decrease in egg production (recording 2248.0±284.20 eggs in control females compared to 420.60±160.64 eggs in rotenone-treated females, respectively) (Fig. 8). Similarly, a significant reduction ($p \leq 0.05$) in the fecundity of 7-day-old females was observed, showing a 41.50% decrease (with 2255.0±225.5 eggs in control females and 1319.0±188.4 eggs in rotenone-treated females) (Fig. 8). Therefore, the application of rotenone inhibits oviposition, either completely or partially, depending on the individual female.

Rotenone's impact on oviposition preference of adult females *C. albiceps* and their eggs hatchability

For assessment of oviposition preference for females of *C. albiceps*, food and smell experimental tubes were provided as choices. Females' oviposition preference was oddly affected by rotenone treatment. The average female fecundity by treated females on food and smell tubes were 220.40 ± 110.74 and 200.20 ± 70.20, respectively, with no significant difference ($p \geq 0.05$). In contrast, the average numbers of eggs deposited by the control females on the food and the smell tubes were 2248.0 ± 284.20 and 0.0 ± 0.0, respectively, with an extremely significant difference ($p \leq 0.001$) between them (Fig. 9).

Eggs laid by females in control group and those treated with rotenone were permitted to undergo their full incubation period and subsequently hatched on the substrate selected by the parent females. The mean percentage hatchability of the eggs laid by treated females was 71.20±5.31, which is significantly lower ($p \leq 0.001$) than those laid by the control ones (93.07± 2.21) (Fig. 10). The selection of the smell tube as an oviposition site by treated females prompted a reduction in offsprings survival with a 48.67% decrease in treated females compared to only 6.93% in control females as illustrated in Fig. 11.

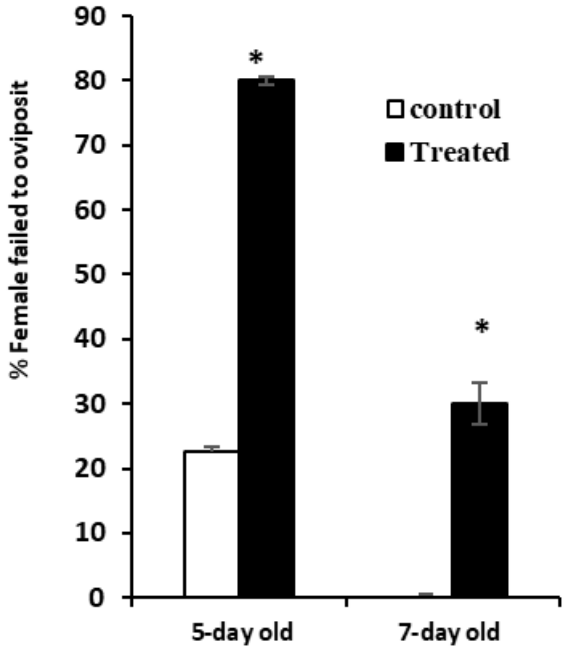

Fig. 7. Percentage of the 5-day-old, and 7-day-old control and rotenone-treated females *C. albiceps* failed to lay eggs. *: statistically significant at $p \leq 0.05$ using Student t-test.

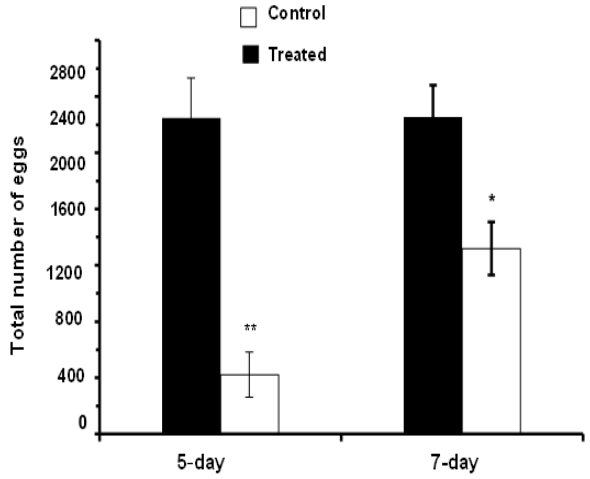

Fig. 8. Mean numbers of eggs laid (±SD) by the 5-day-old**, and control and rotenone-treated females *C. albiceps*. *: statistically significant at $p \leq 0.05$ using Student t-test. **: statistically significant at $p \leq 0.001$ using Student t-test.

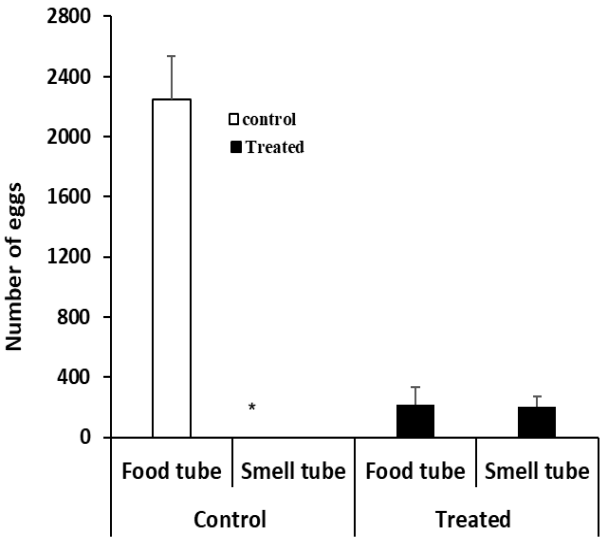

Fig. 9. Mean numbers of eggs laid (±SD) by the control and rotenone-treated females *C. albiceps* on the food and smell tubes. *: statistically significant at $p \leq 0.001$ using Student t-test.

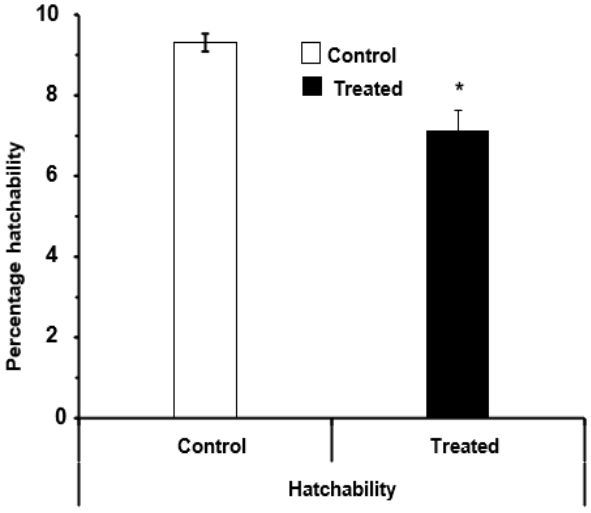

Fig. 10. Mean percentage hatchability (±SD) of eggs laid by the control and rotenone-treated females *C. albiceps*. *: statistically significant at $p \leq 0.001$ using Student t-test.

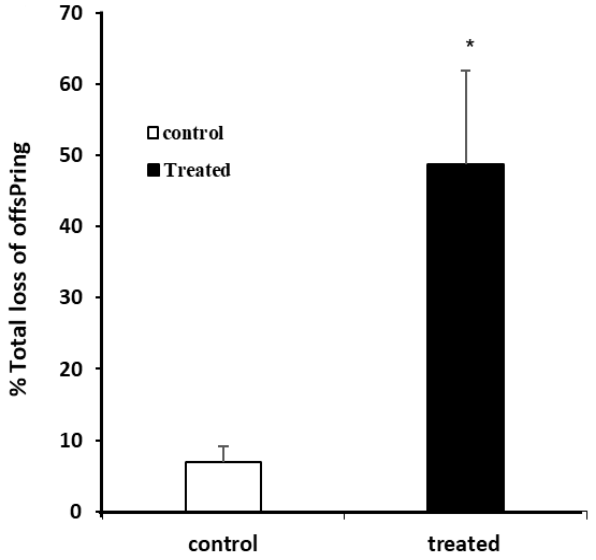

Fig. 11. Percentages of offspring loss (Mean± SD) of the control and the treated groups. *: statistically significant at $p \leq 0.001$ using student t-test.

Discussion

Controlling pests with natural and biodegradable products has been investigated as an alternative to reduce environmental risks and to avoid resistance to chemical insecticides (Regnault-roger et al., 2012; Zhang et al., 2019). Entomological studies using rotenone on dipteran insects were performed to control harmful species such as the warble fly, *Hypoderma spp.* (Diptera: Oestridae) (Ogg 1977) and mosquitoes (Diptera: Culicidae) (Ji et al., 2015). Data collected on the effects of rotenone on different biological aspects of *C. albiceps* is scarce. The current study investigates the feasible alterations induced by rotenone exposure on rate of development, oviposition abilities, and preference of *C. albiceps*, considering its medical importance. Rotenone-treated larvae of *C. albiceps* showed significant decreases in their lengths and weighs, compared to controls, thus it may have an accumulative effect on the development of treated larvae. Corresponding' to the observation of Xavier et al. (2015) on the adult workers of *Apis mellifera* (Hymenoptera: Apidae), the mortality rates of larvae and pupae of *C. albiceps* imply that larvae are more susceptible to rotenone. The elevated survival rates noted in pupae may be attributed to the larvae's ability to metabolize and eliminate the pesticide prior to entering the pupation phase (Oliveira et al., 2009). The stages of oogenesis in *C. albiceps* were similar to those previously stated for other calliphorids: *Lucilia cuprina* (Clift and McDonald, 1973), *Chrysomya. bezziana* (Spradbery and Sands, 1976), *Chrysomya. putoria* (Avancini and Do Prado, 1986), and *Chrysomya. megacephala* (Chaiwong et al., 2012). However, it was cleared out that the period between the second and third stages of egg development in *C. albiceps* lasted longer than other stages, which is consistent with the observation of Adams and Reinecke (1979) on *Cochliomyia hominivorax.*

The third follicle was observed to be separated from the germarium of *C. albiceps* at 80–90 hrs after emergence. No further germarial activity was noticed up to the 7th day. In accordance with the study of Clift and McDonald (1973) on *L. cuprina*, the development of the penultimate follicle is paused at 50 hrs. Furthermore, rotenone exposure affected the stages of oogenesis of female *C. albiceps*, causing developmental retardation at each stage of oogenesis, producing immature eggs with distinct undeveloped chorion. The germarium developmental activity was reduced, which might indicate developmental toxicity. Recently, kumar et al. (2022) reported a significant association between rotenone and developmental inhibition, as well as ovarian abnormality structures on exposure of female *Drosophila melanogaster*. These findings also correspond with the effects of Dichlorodiphenyltrichloroethane (DDT) on *M. domestica*, the effects of precocene on *D. melanogaster* and *Phormia regina* (Beard, 1965; Wilson et al. 1983; Yin et al., 1989). Moreover, the dissected ovaries of treated *C. albiceps* showed a significant decrease in egg load and immature eggs in comparison to controls. This coincides with rotenone effects on *Aphis rumicis* and *Anopheles quadrimaculatus* (Sun, 1945; DeCoursey et al., 1953). Sodium fluoride exposure in *Bombyx mori* and *D. melanogaster* gave similar results (ElGerdes et al., 1971; Chen 2003a, b; khatun et al., 2017). Evidently, rotenone exposure may have the same potential to impair the reproductive success of *C. albiceps*, decreasing egg load and suppressing oviposition. The oviposition preference-offspring performance (P-P) hypothesis predicts how

ovipositing females should distribute the offspring according to their requirements in a heterogeneous environment (Thompson, 1988; Valladares, 1991; Nufio and Papaj, 2004; Ellis, 2008). Previous studies on necrophagous blowflies by Barton-Browne (1962), Hammack (1991), Archer and Elgar (2003) and Charabidze et al. (2015) revealed that insects prefer humid over non-humid media, as their eggs and larvae are very susceptible to desiccation. In the present study, control females preferred to deposit eggs on the food tube as a proper medium for offspring survival, however, rotenone-treated females almost had an equal preference for the food and experimental tubes. Females preferred to deposit eggs on the smell tube, leaving their offspring exposed to desiccation and starvation. This explains the significant reduction of egg hatchability in the experimental smell tube medium. These results suggest that rotenone could affect the flies olfactory and tactile senses as they almost had an equal preference for the food and smell tubes. The impaired oviposition preferences of *C. albiceps* due to rotenone exposure is an added advantage, as the surviving females will not be able to foresee the needs of their offspring, which in turn will destroy the offspring's chance of survival.

Conclusion

Rotenone, as one of the natural plant toxins, was chosen to examine its effects on *C. albiceps*, a fly of medical and veterinary importance. Treatment was found to affect some developmental parameters of *C. albiceps*, delaying development and elevating the mortality rates of larvae and adults. Rotenone also affected the reproduction of the fly, inhibiting the oogenesis process, causing developmental retardation in the structure and length of the follicle, reducing germarium activity, inhibiting egg maturation, and producing immature eggs. Moreover, it inhibited oviposition abilities, decreasing fecundity and egg hatchability *C. albiceps* by impairing females' decision-making abilities, demonstrating that rotenone could be applied as a bioinsecticide to control the *C. albiceps* population. Further studies, should be taken into account to be conducted as possible dose-dependent impact on *C. albiceps*.

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