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RESEARCH ARTICLE

THE ACTIVITY OF *JUNIPERUS PROCERA* STEM EXTRACTS AS PESTICIDES AGAINST THE BLOWFLY *CHRYSOMYA ALBICEPS* (DIPTERA: CALLIPHORIDAE)

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ABSTRACT

The blowfly (*Chrysomya albiceps*) larvae cause cutaneous myiasis in humans/animals. Therefore, the current study aimed to evaluate the pesticidal activity of *Juniperus procera* stem extracts on the 1st instar larvae of *C. albiceps* to minimize their spread. The tested petroleum ether, acetone, and methanol extracts of *J. procera* stem showed toxic effects against the *C. albiceps* larvae, which were mostly dependent on the solvent type and the extract concentration. The larval mortality was found to increase as the concentration of plant extract increased. Petroleum ether extract of *J. procera* stem had a highly toxic effect at the highest concentration (0.4 g/mL) against the larvae (83.3%; LC_{50} = 0.15 g/mL), followed by methanol extract (63.3%; LC_{50} = 0.24 g/mL) and acetone extract (43.3%; LC_{50} = 0.53 g/mL). Acetone extract of *J. procera* stem had a delayed toxic effect on the pupae resulting from the treated larvae. The toxicity of all *J. procera* stem extracts was extended to the adults that resulted from the treated larvae. Larval and pupal durations were affected significantly (P<0.05) by methanol and acetone extracts of *J. procera* stem. All *J. procera* stem extracts exhibited remarkable effects on the fecundity, fertility, and sterility index of *C. albiceps* adult females resulting from larval treatment. Moreover, malformations among pupae and adults of *C. albiceps* were observed after treating the larvae with acetone and petroleum ether extracts of *J. procera* stem. Therefore, *J. procera* has promising efficacy to develop as botanical pesticide for controlling the 1st instar larvae of *C. albiceps*.

INTRODUCTION

A large number of flies belonging to the family Calliphoridae, Muscidae, and Sarcophagidae are vectors to several diseases such as typhoid fever, cholera, tuberculosis, bacillary dysentery, and trachoma virus[1-3]. The main reason for the economic loss of livestock in the world is due to the spread of the fly species *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae) that causes cutaneous myiasis in tropics of the world. *C. albiceps* is known for producing myiasis in humans and animals, and transmitting pathogens

mechanically[4,5]. In Saudi Arabia, cattle (especially sheep and goats) are the main source of animal protein. Several reports on a variety of communicable and non-communicable diseases, which affect the economic production of livestock, are given by the Ministry of Environment, Water and Agriculture of Saudi Arabia, but with very little information about the vectors that cause or carry these diseases[6]. The pest "*C. albiceps*" is common in Southeast Asia, Arabian Peninsula, India, and Africa[7,8]. It had been reported in Jeddah[9], as well as north, center, east, and south of Saudi Arabia[10]. *C. bezziana* and *C. albiceps* are the two main species responsible for causing cutaneous myiasis in Saudi Arabia[11,12]. Myiasis by dipteran larvae causes a nuisance, less fertility/productivity, a decrease in milk production, blindness, muscle damage, and offspring death[13]. To minimize the economic losses for the cattle industry, alternative techniques for population eradication of the flies causing myiasis are needed.

Till now, controlling myiasis has used synthetic pesticides such as spiramycin, amitraz, coumaphos, ivermectin, fenthion, enrofloxacin, and diazinon by dipping methods and topical treatment[14-16]. Moreover, unfavorable effects of the use of synthetic pesticides have been recorded, such as insect resistant, death of natural enemies of pests, toxic to humans and animals, a remnant in milk and meat, as well as causing environmental pollution[17]. So, it is necessary to have alternative insecticides that have some characteristics such as accessible, simple, safe for humans and animals, and leaves no residue in animal products. Plant-derived substances may become the alternative insecticides to synthetic chemicals in control the flies and their larvae that cause myiasis.

The deserts of Saudi Arabia contain large numbers of plants of medicinal importance. There are few studies interested with these plants, most of them have been performed on plant pests and few investigations have been done on medicinal and veterinary pests such as mosquito species. Although there are some references that are interested with flies causing myiasis control among animals in some regions of the world[18-20], very few studies[21,22] are available on the control of flies causing myiasis in Kingdom Saudi Arabia. So, the present study was conducted to test the toxicity of *Juniperus procera* stem extracts on the larvae, pupae, adult, development, and female fecundity of *C. albiceps*.

MATERIAL AND METHODS

Flies rearing

The adult stage of *C. albiceps* was collected in 2019 from Jazan slaughterhouse and transferred to entomology lab (College of Science, Jazan University) to be reared for several generations under laboratory conditions (humidity: 60±10%, temperature: 27±2ºC, and photoperiod: 12 hours dark: 12 hours light). *C. albiceps* was identified according to the key of Shaumar *et al*.[23]. Flies were reared using the previously published method[20] with few modifications. Adults of flies were reared in wooden cages (50×50×50 cm) with wire sides and fed on sucrose solution and milk powder. A plastic plate (15×15 cm) containing fresh beef liver has been placed inside the cage for adult laying their eggs and as a food for the larvae. The 3rd instar larvae were selected and transferred into plastic ponds (20×30×25 cm) containing 400 cm of fine sawdust and left to pupate and the emergence of the adult.

Extraction of plant materials

J. procera (Cupressaceae) was collected from Hashr mountains (17°27'02"N, 43°02'26"E), Jazan Governorate (Saudi Arabia). The stems of the plant were dried in the shadow at lab temperature (maximum 31ºC) and pulverized to powder in a hammer mill. One hundred grams of *J. procera* stem powder were extracted four times with 300 mL of solvents (70% methanol or acetone, or petroleum ether) at lab temperature. After 24 hours, the supernatant was filtrated and dried in a rotary evaporator

at 45ºC. The dry extract was weighed and then kept in the refrigerator at 4°C until the experiments were performed[21].

Experimental bioassay

Larval bioassay

According to the method of Singh and Kaur[24], larval bioassay was performed with some modifications. One day old after hatching from the same egg batch, the 1st instar larvae of *C. albiceps* were used in this bioassay. The larvae were grouped into three groups (20 larvae/group) and reared in separate rearing boxes. Plant extract concentrations were prepared by the two-fold sequential dilution method using distilled water and one drop of Tween 80 to facilitate dissolving of the tested plant extracts in water. Each concentration of the plant extract (0.4, 0.2, 0.1, 0.05, 0.25 g/mL) was put into a bowl and covered with the lid till it was used for the dipping technique. Each group of larvae was enveloped in a voile tissue and softly immersed inside the plant extract solution, while the control group was immersed in distilled water and of one drop Tween 80. The larvae were dipped for 30 seconds and then transferred to the breeding box containing food. The larval mortality was estimated every 24 hours by touching each larva with a paintbrush and those who do not respond are considered dead. Dead larvae and pupae were isolated daily till adult emergence. Malformed larvae, pupae, and adults were isolated daily and placed in tubes containing 70% alcohol and then photographed under the microscope (GX Microscope, GXMXTL3T10, GT Vision Ltd, Suffolk, UK).

Criteria studied

Larval or pupal mortality (%) was estimated as previously described[25] and the mean value was taken.

- Larval mortality (%) = number of dead larvae / number of tested larvae × 100
- Pupal mortality (%) = number of dead pupae / total number of resulting pupae × 100 larval and pupal durations (for each one).
- Adult emergence (%) = a / b × 100, where**:** a = number of emerged adults, b = total number of resulted pupae.

Deformations in larvae, pupae, and adults were estimated by any change in shape, color, size, or failure to progress to the next stage. The growth index was estimated by using the following equation:

- Growth index = A / B, where**:** A = adult emergence (%), B = mean development (days).

Reproductive potential of resulting females

The reproductive efficiency of females resulting from the treated larval was carried out according to Alhuraysi *et al*.[21]. The females that successfully emerge from treated larvae were picked up and transferred with normal adult males (untreated) to the wooden cages (25×25×25 cm). The males and females were fed on powdered milk and sucrose solution for three days, and then the females were transferred separately to the jar (10×10×15 cm) with part of beef liver to lay egg mass. The number of egg/raft (fecundity) after hatching was counted by using a microscope and then the mean value was taken.

The egg-hatchability (fertility) was calculated by the following equation:

- Egg-hatchability (%) = a / b × 100, where: a = total number of hatched eggs, b = total number of eggs laid.

The sterility index (SI) was calculated according to the formula of Toppozada *et al*.[26]:

- Sterility (%) = 100 – [a×b / A×B×100], where: a = number of eggs laid / female in the treatment, b = percentage of hatched eggs in the treatment, A = number of eggs laid / female in the control, B = percentage of hatched eggs in the control.

Statistical analysis

The lethal median concentration (LC_{50}) of larvae was determined based on the mortality data and probit analysis (Excel Program). The larval and pupal durations, as well as female fecundity data were subjected

to statistical analysis by one-way ANOVA followed by Tukey's honest significance test ($P < 0.05$) to determine the difference between the control group and the different treated groups using the statistical package for social sciences (SPSS) software version 19.0 (IBM, Armonk, NY, USA).

RESULTS

Yield of tested plant extracts in different solvents

The obtained dry weight of *J. procera* stem extract was varied from one solvent to another, and methanol extract of *J. procera* stem produced a higher weight (16.1 g/100 g of *J. procera* stem) than acetone (4.6 g/100 g of *J. procera* stem) and petroleum ether (3.1 g/100 g of *J. procera* stem) extracts.

Toxic effects of methanolic, acetone, and petroleum ether extracts of *J. procera* stems

Table (1) indicated the toxicity of methanol, acetone, and petroleum ether extracts of *J. procera* (stem) on the larvae, pupae, and adults of *C. albiceps*. The mortality percent of larvae was found to increase mostly as the concentration of the plant extract increased; petroleum ether extract had a highly toxic effect (83.3%) against the larvae at the highest concentration (0.4 g/mL), followed by methanol extract (63.3%) and acetone extract (43.3%). It was also found that all plant extracts had no toxic effects on pupae, except for the two highest concentrations (0.4 and 0.2 g/mL) of acetone extract (29.4-38.8%) and the methanol extract at 0.1 g/mL (5.5%) compared with the control group (0.0%). Moreover, the methanol extract of plant stem at 0.1 g/mL and the two highest concentrations of acetone extract reduced the adult emergence percent to 94.5%, 70.6%, and 61.2%, respectively, compared with the control group (100%). The methanol, acetone, and petroleum ether extracts of plant stem had a long-lasting lethal effect on the adult at all used concentrations. Some pupal malformations were observed at the two highest concentrations of plant acetone extract (30.0 and 21.4%, respectively). Petroleum ether extract of plant stem also caused some percentages of malformation between adults developed from treated larvae (Figure 1); there was an increase in the percentages of malformed adults as the concentration of plant extract increased, it recorded 50.0%, 38.8%, and 19.2% at plant extract concentrations of 0.4, 0.2, and 0.1 g/mL, respectively.

The results in Table (2) and Figure (2) indicated that the LC_{50} values were arranged as follows: petroleum ether < methanol < acetone. In general, larvicide evaluation of the tested plant extracts in petroleum ether, methanol, and acetone against *C. albiceps* larvae revealed them to possess high, moderate, and low larvicidal activity, respectively, with LC_{50} values ranging from 0.15-0.53 g/mL.

Effect plant extracts on larval and pupal development

Results in Table (3) revealed that the methanol extract of plant stem caused a significant (P<0.05) prolongation in the mean larval period at all used concentrations; where it recorded 6.70± 0.97, 7.08±0.97, 7.55±1.49, 7.26±0.43, and 7.58±1.68 days at 0.4, 0.2, 0.1, 0.05, and 0.025 g/mL, respectively, *versus* 6.05± 0.61 days for the control group. Moreover, the mean pupal duration especially at the highest concentrations of plant methanol extract (0.4 and 0.2 g/mL) decreased significantly (P<0.05) to 5.36±0.48 and 4.60±0.48 days, respectively, *versus* 5.78± 0.77 days for the control group. The growth index was not affected as compared with the untreated group. On the other hand, the control larvae reached the pupal stage in 6.54±0.89 days, while this duration was prolonged significantly (P<0.05) to 6.82±0.97 and 7.24±0.96 days at the lowest concentrations (0.05 and 0.025 g/mL, respectively) of plant acetone extract. A significant increase (P<0.05) in the mean duration of pupae at all concentrations of the plant acetone extract was observed (from 5.51±0.78 days at the lowest concentration

Table 1: The toxic effects of methanol, acetone, and petroleum ether extracts of *Juniperus procera* (stem) on different stages of *Chrysomya albiceps.*

Plant extracts	Concentration (g/mL)	Larval mortality		Pupation		Pupal mortality		Malformed pupae		Total larval and pupal mortality		Adult emergence (%)	Adult mortality (%)	Malformed adult (%)
		No.	%	No.	%	No.	%	No.	%	No.	%			
Methanol	0.4	38	63.3	22	36.7	0	0.0	0	0.0	38	63.3	100	81.8	0.0
	0.2	31	51.6	29	48.4	0	0.0	0	0.0	31	51.6	100	75.8	0.0
	0.1	24	40.0	36	60.0	2	5.5	0	0.0	26	43.3	94.5	50.0	0.0
	0.05	18	30.0	42	70.0	0	0.0	0	0.0	18	30.0	100	28.6	0.0
	0.025	12	20.0	48	80.0	0	0.0	0	0.0	12	20.0	100	29.1	0.0
Acetone	0.4	26	43.3	34	46.7	10	29.4	3	30.0	36	60.0	70.6	79.1	0.0
	0.2	24	40.0	36	60.0	14	38.8	3	21.4	38	63.3	61.2	40.9	0.0
	0.1	25	41.6	35	48.4	0	0.0	0	0.0	25	41.6	100	54.3	0.0
	0.05	21	35.0	39	65.0	0	0.0	0	0.0	21	35.0	100	23.1	0.0
	0.025	15	25.0	45	75.0	0	0.0	0	0.0	15	25.0	100	35.5	0.0
Petroleum ether	0.4	50	83.3	10	16.7	0	0.0	0	0.0	50	83.3	100	20.0	50.0
	0.2	42	70.0	18	30.0	0	0.0	0	0.0	42	70.0	100	27.7	38.8
	0.1	34	56.6	26	43.4	0	0.0	0	0.0	34	56.6	100	26.9	19.2
	0.05	14	23.3	46	76.6	0	0.0	0	0.0	14	23.3	100	23.9	0.0
	0.025	10	16.6	50	83.3	0	0.0	0	0.0	10	16.6	100	16.0	0.0
Control	0.0	2	3.3	58	96.7	0	0.0	0	0.0	2	3.3	100	1.7	0.0

No.: number

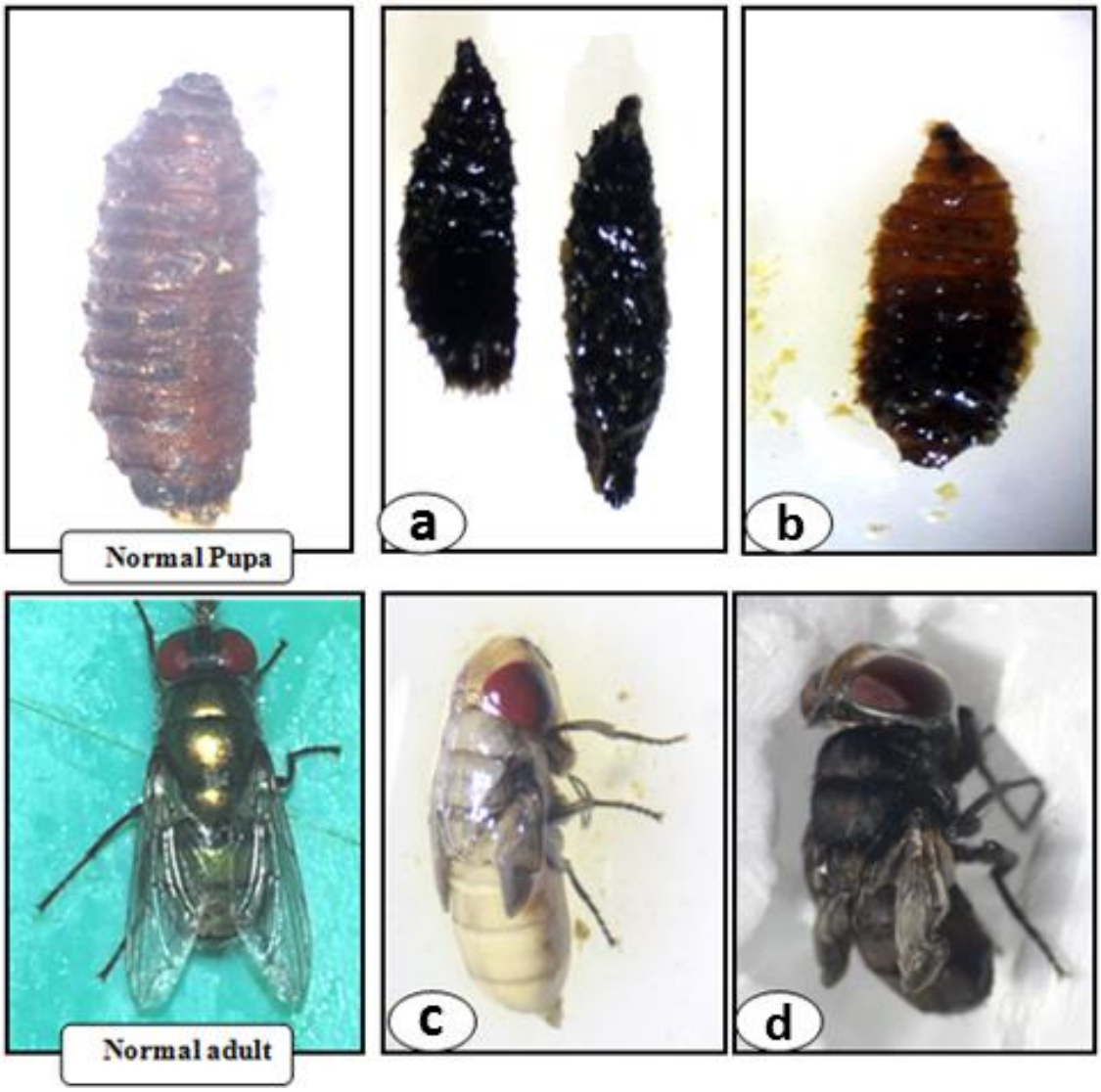

Figure 1: Morphological abnormalities occurred after treatment of 1st larvae of *Chrysomya albiceps* with *Juniperus procera* plant extracts (×160). (**a**) Dead puparium with shrunk and black colored. This feature was induced by plant acetone extract (0.4 and 0.2 g/mL). (**b**) Dead puparium with swollen posterior dark appearance resulted from larvae treated with plant acetone extract (0.4 and 0.2 g/mL). (**c**) Dead deformed adult with small wings, white (discolored) body colored, and small eyes. This feature was induced by petroleum ether extract of plant stem (0.4, g/mL). (**d**) Dead deformed adult with shrink wings and dark (black) body colored resulted from the treatment of the larvae with petroleum ether extract of plant stem (0.2 and 0.1 g/mL).

Table 2: Relative efficiency of *Juniperus procera* stem extracts against *Chrysomya albiceps* larvae**.**

Solvent	LC_{50} (g/mL)	Slope (b)	Correlation coefficient (R^2)
Methanol	0.24	106.2	0.859
Acetone	0.53	34.3	0.504
Petroleum ether	0.15	170.3	0.798

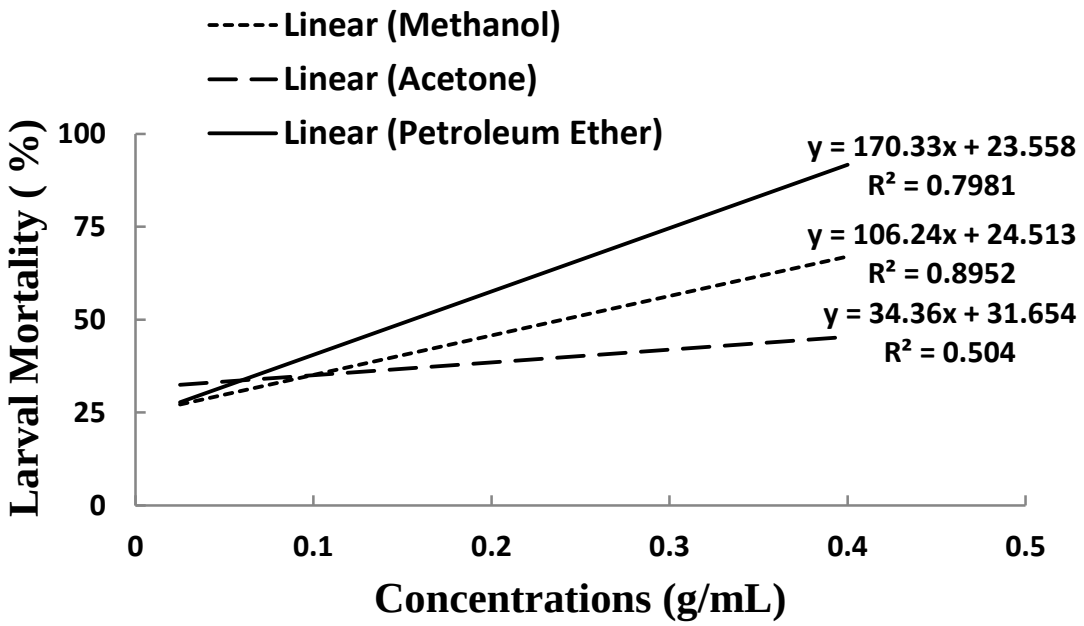

Figure 2: Insecticidal activities of methanol, acetone, and petroleum ether extracts of *Juniperus procera* stem against the 1st larvae of *Chrysomya albiceps*.

Table 3: Larval and pupal developmental periods (days) of 1st instar larvae of *Chrysomya albiceps* treated with methanol, acetone and petroleum ether extract of *Juniperus procera* stem.

Plant extracts	Concentration (%)	Larval duration (Mean±SD)	Range	Pupal duration (Mean±SD)	Range	Total larval and pupal duration±SD	Growth Index
Methanol	0.4	6.70±0.97^{b}	6-8	5.36±0.48^{b}	5-6	12.06±1.45^{a}	8.3
	0.2	7.08±0.97^{c}	6-9	4.60±0.48^{a}	4-5	11.68±1.45^{a}	8.5
	0.1	7.55±1.49^{e}	6-9	6.07±0.97^{d}	6-6	13.62±2.46^{c}	7.0
	0.05	7.26±0.43^{d}	6-9	5.66±0.86^{c,d}	5-7	12.92±1.29^{b}	7.7
	0.025	7.58±1.68^{e}	6-10	5.60±0.88^{c}	5-7	13.18±2.56^{b}	7.6
Control	0.0	6.05±0.61^{a}	6-6	5.78±0.77^{c}	5-7	11.83±1.38^{a}	8.4
Acetone	0.4	6.44±0.45^{a}	6-7	6.83±1.02^{e}	5-8	13.27±1.47^{d}	5.3
	0.2	6.46±0.46^{a}	6-7	6.59±0.65^{d}	5-7	13.05±1.11^{c,d}	4.7
	0.1	6.68±0.94^{a,b}	6-8	6.02±0.99^{c}	5-7	12.70±1.93^{c,b}	7.8
	0.05	6.82±0.97^{b}	6-8	5.53±0.49^{b}	5-6	12.35±1.46^{b}	8.1
	0.025	7.24±0.96^{c}	6-8	5.51±0.78^{b}	5-7	12.75±1.72^{b}	7.8
Control	0.0	6.54±0.89^{a}	6-8	5.11±0.31^{a}	5-6	11.65±1.20^{a}	8.6
Petroleum ether	0.4	8.28±0.77^{a}	8-8	5.10±0.30^{a}	5-6	13.38±1.07^{a}	7.5
	0.2	8.24±0.49^{a}	8-8	5.39±0.47^{b}	5-6	13.63±0.96^{a}	7.3
	0.1	8.38±0.48^{a}	8-9	5.40±0.48^{b}	5-6	13.78±0.96^{a}	7.3
	0.05	8.40±0.47^{a}	8-8	5.41±0.40^{b}	5-6	13.81±0.92^{a}	7.2
	0.025	8.36±0.49^{a}	8-8	5.44±0.68^{b}	5-7	13.80±1.17^{a}	7.2
Control	0.0	8.35±0.47^{a}	8-9	5.46±0.49^{b}	5-6	13.81±0.96^{a}	7.2

Means followed by a different letter(s) in the same column, for each plant extract and its control, were different significantly (P<0.05) from each other. SD: standard deviation.

to 6.83±1.02 days at the highest concentration) as compared with the control group (5.11±0.31 days). Larvae and pupae growth index was greatly affected by acetone extract at the two highest concentrations (0.4 and 0.2 g/mL); where it recorded 5.3 and 4.7, respectively, *versus* 8.6 for the control group. Petroleum ether extract of plant stem did not affect significantly (P>0.05) the mean pupal duration at all used concentrations, except at the highest concentration (0.4 g/mL); where the pupal duration was shortened significantly (P<0.05) to 5.10±0.30 days versus 5.46±0.49 days for the untreated group. At all concentrations, the growth index was not affected by petroleum ether extract of plant stem.

Effect of plant extracts on fecundity, fertility, and sterility index

As summarized in Table (4), the plant methanol extract exerted a profound reducing action on female fecundity at all used concentrations; the fecundity was 50.0±0.0, 61.0±0.0, 92.5±24.0, 95.3±31.8 and 143.0±32.7 eggs/♀ at 0.4, 0.2, 0.1, 0.05 and 0.025 g/mL, respectively, while for the control group it was 175.0±17.3 eggs/♀. At the three highest concentrations (0.4, 0.2, and 0.1 g/mL) of plant methanol extract, the hatchability rate was reduced to 18.0, 44.2, and 76.2%, respectively, compared with 99.0% for the control group. The sterility index was increased by increasing the concentration of plant methanol extract; it recorded 94.8% and 22.4% at the highest and lowest concentration (0.4 and 0.025 g/mL, respectively). A significant decrease (P<0.05) in the mean number of eggs laid at all concentrations was also observed by the plant acetone extract; it recorded 47.0±0.0 and 151.6±41.7 eggs/♀ at the highest (0.4 g/mL) and lowest (0.025 g/mL) concentration, respectively, compared to 187.5±7.6 eggs/♀ for the control group. The hatchability percent decreased as the concentration of the plant acetone extract increased; the hatchability percent recorded 0.0%, 66.6%, and 89.4% at 0.4, 0.2, and 0.1 g/mL of plant extract, respectively, compared with 99.3% for the untreated group. The sterility index was highly affected at all concentrations of the plant acetone extract; it recorded 100 and 22.2% at the highest and lowest concentrations (0.4 and 0.025 g/mL, respectively). As for the plant petroleum ether extract, it had a significant deficiency effect on the average egg-laying by females resulting from the treatment of larvae. The average number was 37.0±0.0, 72.5±35.0, 120.0±38.4, 147.1±36.3, and 150.0±33.3 eggs/♀ at 0.4, 0.2, 0.1, 0.05, and 0.025 g/mL of the plant petroleum ether extract, respectively, *versus* 208.0± 29.6 eggs/♀ for the control group. The hatchability percent was 0.0, 77.2, 87.5, 94.1, and 95.1% at 0.4, 0.2, 0.1, 0.05 and 0.025 g/mL of the plant petroleum ether extract, respectively, *versus* 97.8% for the control group. The sterility index was increased by increasing the concentration of the petroleum ether extract of plant stem; it recorded 100.0, 72.5, 48.4, 32.0, and 29.9% at the concentrations: 0.4, 0.2, 0.1, 0.05, and 0.025 g/mL of the plant extract,, respectively, *versus* 0.0% for the control group.

DISCUSSION

The current study described in detail the impact of methanol, acetone, and petroleum ether extracts of *J. procera* stem on the development of *C. albiceps* larvae by using the dipping method. The choice of dipping techniques depends on the method used to control ectoparasites in cattle. The active constituents of the plant extracts may penetrate the body of larvae through ingestion or the cuticle. Previous researches indicated that the plant extracts can pass through the gut of larvae and destroy the epithelial cell lining and kill them[27]. Development inhibiting activity reduced pest damage effect even without killing the pest. Furthermore, in the long run, populations are reduced through disrupted metamorphosis or reduced fecundity[28]. The differences in the toxicity of chemical plant compounds on

target species depend on the part of the plant to be extracted and the solvent used for extraction, in addition to other differences due to species responses, the developmental stages in the specific extract, the plant geographic origin, photosensitivity to compounds in the extract, and the influence on development and reproductively[21,29]. Furthermore, crude or partially purified plant extracts have been shown to be less expensive and highly effective in controlling flies than the pure compounds[30,31].

Table 4: Effect of *Juniperus procera* (stem) methanol, acetone, and petroleum ether extracts on sterility index, fertility, and fecundity of female *Chrysomya albiceps*.

Plant Extracts	Concentration (g/mL)	No. of tested females	Eggs laid		Hatched eggs		Sterility index (%)
			Total	Mean±SD	Total	%	
Methanol	0.4	1	50	50.0*	16	18.0	94.8
	0.2	1	61	61.0*	27	44.2	84.4
	0.1	2	185	92.5±24.0[a]	141	76.2	59.3
	0.05	6	575	95.3±31.8[a]	550	95.6	47.4
	0.025	9	1287	143.0±32.7[b]	1212	94.1	22.4
Control	0.0	15	2625	175.0±17.3[c]	2600	99.0	0.0
Acetone	0.4	1	47	47.0*	0	0.0	100
	0.2	5	594	118.8±34.6[a]	396	66.6	57.5
	0.1	4	445	111.3±32.1[a]	398	89.4	46.6
	0.05	6	850	141.6±29.9[b]	800	94.1	28.4
	0.025	9	1365	151.6±41.7[a,b]	1305	95.6	22.2
Control	0.0	12	2250	187.5±27.6[c]	2235	99.3	0.0
Petroleum ether	0.4	1	37	37.0*	0	0.0	100
	0.2	2	145	72.5±35.0[a]	112	77.2	72.5
	0.1	5	600	120.0±38.4[b]	525	87.5	48.4
	0.05	8	1177	147.1±36.3[c]	1108	94.1	32.0
	0.025	12	1800	150.0±33.3[c]	1713	95.1	29.9
Control	0.0	15	3120	208.0±29.6[d]	3053	97.8	0.0

Means followed by a different letter(s) in the same column, for each plant extract and its control, were different significantly (P<0.05) from each other. No.: number; SD: standard deviation. *Number of eggs laid for one female.

The results of our study recorded that all the stem extracts of *J. procera* in various solvents had toxic effects against *C. albiceps* larvae, and the toxic effect was mostly depends on the extract concentration and the type of solvent used in the extraction process. The death rate of larvae increased with increasing the concentration of the extract in most cases. Based on LC_{50} values, the data of our results indicated that the toxic effect of the plant petroleum ether extract was more than the plant methanol extract and the plant acetone extract. Many different plant extracts other than those used in the current study have been examined on various species of Calliphoridae flies by several authors worldwide[32-36]. In the present study, petroleum ether and methanol extract showed a good larvicidal effect against 1st instar larvae of *C. albiceps* with LC_{50} 0.15 and 0.24 g/mL, respectively. The essential oil of *J. procera* had also demonstrated varying degrees of larvicidal activity against *An. Arabiensis*; its LC_{50} value was 24.65 mg/L[37]. The toxic effect of diethyl ether and hexane extract of *Artemisia herba* caused 100% larval mortality against the 3rd instar larvae of

C. albiceps at the concentration of 2.93 and 2.95 g/mL, respectively, by using dipping technique, while hexane and diethyl ether extract of *A. monosperma* caused 73.3% and 13.3% larval mortality at the concentrations of 7.7 and 2.5 g/mL, respectively[38]. Such results may be comparable with the results of the current study on the larvicidal activity for *J. procera* plant species against *C. albiceps*, whereas the petroleum ether, methanol, and acetone extracts of plant stem caused 83.3%, 63.3%, and 43.3% larval mortality at the highest concentration (0.4 g/mL). The existence of variations in toxicities of phytochemical compounds on target species confirms the previously mentioned suggestion of Fraternale *et al*.[29]. Using crude petroleum ether, chloroform, ethyl acetate, and methanol extracts of *Azadirachta indica* against the 3rd instar larvae of *C. bezziana* showed that all extracts in the dipping method had toxic effects on the larval stage; the highest mortalities were recorded in methanol extract followed by chloroform, petroleum ether, and ethyl acetate extracts with LC_{50} values of 1.07, 1.7, 3.39, and 4.9 g/100 mL, respectively[24]. However, the present study showed that the highest mortalities were recorded in the petroleum ether extract of *J. procera* stem with LC_{50} 0.15 g/mL. The petroleum ether leaves extract of *Artemisia absinthium* at 0.4 g/mL caused 100% larval mortality on *C. albiceps* with LC_{50} of 0.11 g/mL[21]. Therefore, the test methods, differences between species, and exposure time to the test materials may be responsible for the differences among the obtained results.

In general, the plant methanol extract used in the current study induced a significant increase in the growth period of larvae at all concentrations, while the plant acetone extract induced a significant increase in the growth period of pupae at all concentrations. In addition, the plant acetone extract at all concentrations and the plant methanol extract at the lowest three concentrations induced a significant prolongation in the total developmental period (larvae and pupae), while the petroleum ether extract of pant stem had no significant effect on the total development period (larvae and pupae). Therefore, the prolongation of development was dependent mainly on the solvent used in extraction and secondarily on the extract concentration. These results are in agreement with previous reports[21,28,39] using an aqueous extract of *Pouteria sapota* leaf on the post-embryonic development of *Chrysomya putoria*, 10% concentration of neem seed and neem seed kernel powders against 1st, 2nd, and 3rd instar larvae of the blowfly, *Chysomya chloropyga*, and petroleum ether, acetone, and ethanol extracts of *A. absinthium* leaves against the 1st instar larvae of *C. albiceps*. However, Chil-Núñez *et al*.[36] found the essential oil of *Ocimum sanctum* reduced the duration of immature stages and had a direct effect on the viability of *C. putoria* fly (LC_{50} = 7.47 mg/mL). The most significant hormonal changes occur in the pupal period in the holometabolous insects. The substances extracted from plants and tested for insects control could modify specific physiological processes, such as the neurohormonal and the endocrine control of insect growth[40].

In the present study, *J. procera* acetone extract had delayed toxic effects extended to the pupae. This extract also caused a decrease in the percentage of adult insects that emerged from the pupae resulting from the treated larvae. The emergence of the adult phase was dependent on the concentration and type of extract. These results are similar to the earlier results of Mohamed *et al*.[19], using *Commiphora molmol* and *Balanites aegyptiaca* against the blowfly "*Lucilia sericata*". The seed powder of neem at a concentration of 10% also inhibits the adult emergence[28]. In addition, the percentage of adult emergence of *L. sericata* was markedly decreased after treatments with acetone extracts from the tree of heaven *(Ailanthus altissima*), dill *(Anethum graveolens*) and coriander *(Coriandrum sativum*)[34]. Plant extracts that accelerate or prolong the larval duration likely caused a hormonal imbalance in the

organism[41], which probably affected the normal development of the structure that facilitated ecdysis in adults. In the current study, the results indicated that the toxic effects of the tested plant extracts were extended to the adult stage causing mortality ranging from 16.0% to 81.8%. The plant extracts that induced more than 50% mortality in the produced adults at the highest concentration (0.4 g/mL) were methanol and acetone extracts. At the time of this study, there are no previous studies were performed on the impact of plants extracts on survivorship of the resulted adults from treatment and growth index for Calliphoridae species, except the study of Ajayi and Muse[28] that tested neem seed and neem seed kernel powders against the survival and the longevity of blowfly "*Chrysomya chloropyga*". In addition, Alhuraysi *et al*.[21] concluded that the toxic effect of acetone and methanol extracts of *A. absinthium* against *C. albiceps* larvae was extended to pupae, and all plant extracts had delayed toxic effect on the adults.

Almost all plant extracts and their concentrations reduced significantly the fecundity and increased the sterility index of females developed from treated larvae as compared with the untreated control. The fecundity and sterility index were also dependent on the used solvent in plant extraction and the extract concentration. Furthermore, a noticeable decrease was observed in the percentage of eggs hatched by females resulting from treating larvae with petroleum ether extract of plant stem. In the present study, acetone and petroleum ether extract caused some distortions in an immature and mature stages. The developmental abnormalities induced by the plant extracts may be due to an interference with the neuro-endocrine control of ecdysis[42]. Three endocrine glands in the immature stage (the corpus cardiacum, the corpus allatum, and the prothoracic gland) are known to be responsible for releasing neurohormones essential for differentiation growth and development. It has been shown that plant compounds cause a gradual degradation of all larval endocrine glands[43]. Deterioration in morphological structures indicates a general defect of the neuro-endocrine system leading to prolongation of larval and pupal life. Some abnormalities in larvae and pupae after treating *L. sericata* with commercial oils from *B. compestris*, *A. graveolens*, *T. foenum*, and *R. sativus* were observed[32]; the larval deformities include crooked, small, shrunken, and dark-colored larvae, while the pupal deformities include larviform, small, and fissured larvae, and the deformities of adult include young adults and deformed legs, wings, and abdomen. The *C. molmol* oil and *B. aegyptiaca* alcoholic extract induced morphological abnormalities in *L. sericata* like shrunken and small larvae[19]. Varying degrees of morphogenetic abnormalities were recorded in immature stages of *C. bezziana* (shrunk larva and dark colored, larval-pupal intermediate) when larvae were treated with methanol, petroleum ether, chloroform, and ethyl acetate extracts of *A. indica*[24]. The essential oil extracted from *Ocimum sanctum* with 5% and 10% concentrations exhibited morphological alterations of *C. putoria* adults[36]. The larval and pupal deformation was also recorded when testing methanol and acetone extract of *A. absinthium* on *C. albiceps* larvae[21].

In conclusion, the plant extracts used in the present study not only killed larvae when used at high concentrations, but also inhibited metamorphosis and caused morphological deformities at sub-lethal concentrations. These extracts can inhibit the emergence of the adult. Further studies will be conducted to test the efficacy of these plant extracts on field strains, which may lead to the future development of effective natural control against blowflies and may be incorporated with other pest control programs.

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CONFLICT OF INTEREST

The authors have no potential financial conflict of interest.

AUTHORS' CONTRIBUTIONS

AMSA: Master's student responsible for the project, participated in the planning and execution of bioassays, data analysis, and writing of the manuscript. TMYS: Project supervisor; guided the data collection and reviewed the manuscript. UMAE: Assistant project supervisor, assisted in the setup and evaluation of experiments in the laboratory and in the analysis of the data. All authors have read and approved the manuscript.

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نشاط مستخلصات ساق نبات العرعر "*Juniperus procera*" كمبيدات حشرية ضد ذُبابة "*Chrysomya albiceps*" (رتبة: Diptera ، فصيلة: Calliphoridae)

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تسبب يرقات ذبابة "*Chrysomya albiceps*" النغف الجلدي في البشر والحيوانات. لذلك، هدفت الدراسة الحالية إلى تقييم نشاط مستخلصات ساق نبات العرعر "*Juniperus procera*" كمبيدات حشرية ضد يرقات الطور الأول لذبابة "*Chrysomya albiceps*" لتقليل انتشارها. وقد أظهرت مستخلصات الإثير البترولي والأسيتون والميثانول لساق نبات العرعر تأثيرات سامة ضد يرقات ذبابة "*Chrysomya albiceps*"، والتي كانت تعتمد في الغالب على نوع المذيب وتركيز المستخلص. وقد وجد أن معدل وفيات اليرقات (%) يزداد مع زيادة تركيز المستخلصات النباتية. وكان لمستخلص الإثير البترولي لساق نبات العرعر تأثير شديد السمية عند أعلى تركيز (0.4 جم/مل) ضد اليرقات (نسبة وفيات 83.3%؛ التركيز نصف المميت = 0.15 جم/مل)، يليه مستخلص الميثانول (نسبة وفيات 63.3%؛ التركيز نصف المميت = 0.24 جم/مل) ومستخلص الأسيتون (نسبة وفيات 43.3%؛ التركيز نصف المميت = 0.53 جم/مل). وكان لمستخلص الأسيتون لساق نبات العرعر تأثير سام متأخر على العذارى الناتجة عن معالجة اليرقات، كما امتدت سمية جميع مستخلصات ساق نبات العرعر إلى الحشرات البالغة التي نتجت عن معالجة اليرقات. وتأثرت فترات اليرقات والعذارى بدلالة إحصائية ($P<0.05$) بمستخلصي الميثانول والأسيتون لساق نبات العرعر. وأظهرت جميع المستخلصات تأثيرات ملحوظة على إنتاجية وضع البيض والخصوبة والعقم في إناث الأطوار البالغة الناتجة عن معالجة اليرقات. علاوة على ذلك، لوحظ وجود تشوهات في العذارى والأطوار البالغة الناتجة عن معاملة اليرقات بمستخلصات الأسيتون والإثير البترولي لساق نبات العرعر. لذلك، فإن لمستخلصات ساق نبات العرعر فعالية واعدة لتطويرها كمبيدات نباتية للسيطرة على يرقات الطور الأول من ذبابة "*Chrysomya albiceps*".