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Efficacy of some plant wastes and cigarette filter residues as alternative agents for controlling *Culex pipiens* (Diptera: Culicidae)

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Abstract
Mosquito-borne diseases are among the most common public health problems in the world, especially in developing countries. The idea of evaluating certain agricultural and industrial waste products of natural origin as larvicidal agents against mosquitoes is both good and unconventional. Four agricultural waste extracts (Apricot kernels, Lipton packet tea, date palm kernels, and burnt rice straw) and one industrial waste extract (cigarette filters) were evaluated as insecticides against 3rd the larval instar of *Culex pipiens In vitro* and their effect on egg laying and hatching rates. According to the current result, all plant and industrial waste extracts had larvicidal activity, and cigarette filters (100% mortality) and Lipton packet tea (97%) were most effective, followed by date palm kernels (90%), burnt rice straw (84%), and apricot kernel extracts (79%), whereas the LC_{50} values were 528.14, 582.42, 815.5, 976, and 1080.50 ppm with 2000 ppm after 24 h, respectively. Data showed that cigarette filters and apricot kernel extracts were more attractive media for female oviposition, and cigarette filters and date palm kernel extracts had significant effects on the decreasing hatching rate (12.42% and 24.58%). Cigarette filters, Lipton packet tea, and date palm kernels can be used safely and efficiently to control Cx. pipiens larvae.

Keywords: Larvicidal activity, plant waste extracts, *Culex pipiens*, cigarette filters

1. Introduction

Infectious diseases spread by mosquitoes pose a serious threat to the world's population at the present time. There are many mosquito-borne diseases, such as malaria, dengue fever, yellow fever, filariasis, Japanese encephalitis, and Zika [1, 2]. Some reports indicate that more than 1 million people die each year as a result of mosquito-borne diseases worldwide, affecting more than 700 million people [3]. Therefore, control of these vector-borne is a critical step to take.

One of the main methods of controlling vector populations is the use of insecticides [4, 5]. It is considered the most effective solution for controlling these pests, but most mosquitoes have developed resistance to most groups of pesticides, which hinders the effectiveness of insecticide-based control measures and thus the spread of transmitted diseases.

As a result, it is imperative to find non-chemical methods to control a group of arthropods that act as vectors of human disease [6] in a safer, more natural, and sustainable way that does not affect the environment. Research continues, and there is growing interest in natural phytochemicals because they are safer and more efficient than synthetic insecticides. Therefore, we focus on identifying which plants, particularly those that have not been researched or worked on, such as plant waste, are expected to have higher concentrations of bioactive phytochemicals.

Plants are one of the gifts of nature that can treat a variety of human disorders [7, 8]. Also, plant extracts contain broadly effective antimicrobials along with their industrial and multi-industry scope [9-11]. Use some agricultural waste materials to reduce the cost of producing natural pesticides, antimicrobial agents, and other beneficial agricultural products [12-14].

Cigarette butts (CBs) are among the most common types of litter in the world [15] and result in approximately 4.5 trillion cigarettes being littered each year. CB consists of some tobacco fibers, ash, and about 30% filter, along with some chemical additives. Tobacco leaves naturally contain a wide range of compounds, including nicotine and sugar esters [16].

In addition to many other characteristics, such as cigarette size, tobacco rod weight, wrapping paper porosity, and filter type [17], cigarettes may contain approximately 10% by weight of additives known to increase toxicity [18]. The hope that CB wastes can be used in mosquito control is largely supported by the efficacy of nicotine and its derivatives against a variety of insect vectors, including mosquitoes [19, 20].

In this study, we aimed to evaluate the larvicidal activity of some plant residues, such as apricot kernels, Lipton-packet tea, date palm kernels, and burnt rice straw, with the effect of cigarette filter residues as alternative agents against *Culex pipiens* larvae of the West Nile virus vector. The effect of these materials on obstructing the female oviposition was also conducted.

2. Materials and methods

2.1 Plant materials and extraction

The Apricot kernels, Lipton packets of tea, and date palms were purchased from the supermarket (Fig. 1). The rice straw plants were obtained from rice fields in the North Delta, Egypt (Fig. 1). The air-dried Apricot and date palm kernels and Lipton packet teas were washed thoroughly in tap water and dried under indoor conditions at room temperature (27±2 °C) for 11 days. The air-dried materials were powdered separately using a commercial electrical blender. For the extraction, the plant materials were extracted in water (aqueous extraction). Initially, the powder of 10 g of each plant waste was soaked in 200 ml of water for 48 h and filtered through Whatman filter paper No. 1. While the cigarettes consist of a filter for inhaling burnt tobacco (smoke) and a part of the tobacco wrapped inside a thin piece of paper to be the largest part of the cigarette (Fig. 1). In most cigarettes, specifically Marlboro cigarettes, after the smoking process, the cigarette is thrown out as cigarette butts with 1 to 2 cm of unburned tobacco and the cigarette butt filter. Cigarette butt filters were collected, weighed, and soaked in 100 ml of water for 48 h.

2.2 Mosquitocidal bioassay

2.2.1 Mosquito colony

Culex pipiens larvae were obtained from the Insect Breeding Laboratory, Department of Insects, Faculty of Science, Benha University, Egypt, and were used in all research experiments. Larvae were reared in tubs (25 x 20 x 10) filled with 2 L of dechlorinated tap water, Tetramin® fish meal, and dog biscuit powder every 2 days under laboratory conditions (27±2 °C, 75–80% RH, and 12:12 h (L/D photoperiod). When *Cx. pipiens* pupae became adults, they were collected and placed in cages measuring 30x30x30 cm in insect cages, and then transferred to cups measuring 12x10x7 cm. An 8–10% sucrose solution was given as food to adults. After 48 hours of blood feeding, egg bowls filled with distilled water were placed in adult cages. Regularly, egg rafts were collected and placed in white enamel dishes filled with 1 to 1 liter of dechlorinated water. Larvae and adults were exposed to identical laboratory conditions [21].

2.2.2 Larvicidal activity

According to WHO [22], plant material extracts were investigated for their effectiveness against the third larvae of *Cx. pipiens*. The concentrations of plant waste materials and cigarette butt filters (125, 250, 500, 1000, 1500, and 2000 ppm) were performed [22]. The control groups were treated with water only. Twenty larvae were placed in a glass beaker holding 250 ml with various concentrations. Only water was used to treat the experiment and the control group. Five times, the experiments were run. Mortality rates for *Cx. pipiens* larvae was measured 24, 48 h after treatment (PT) at 27±2 °C and 80% relative humidity.

2.2.3 Oviposition inhibition

Twenty adult females and adult males were collected in wooden cages measuring 30x30x30 cm according to WHO protocol [22]. Adults were allowed to starve for one day after being fed with a 10% sugar solution for three days through the cotton pads. On the fifth day, a Syrian hamster was given to starving females for a blood meal. Plant waste extracts were applied inside the cage as follows: (1) 500 ppm, (2) 1000 ppm and (3) tap water (no concentration) in plastic cups that have 500 ml from the previous solutions. The Plant waste concentrations and tap water cups were placed in the corner of the cage for oviposition. Above the waterline, females lay rafts of eggs. The number of eggs in each egg raft was counted using stereomicroscopes. According to Hassan *et al*. [23], the eggs were divided into two groups: hatched and non-hatched. The following equation, which is based on the work of Sheikh [24], was used to calculate the hatching eggs. The percentage of eggs that hatch is equal to A/B100, where A is the total number of eggs hatched and B is the total number of eggs deposited, as well as control substrate egg samples. The hatching of the eggs was observed 48 h after their placement on the surface of the cups.

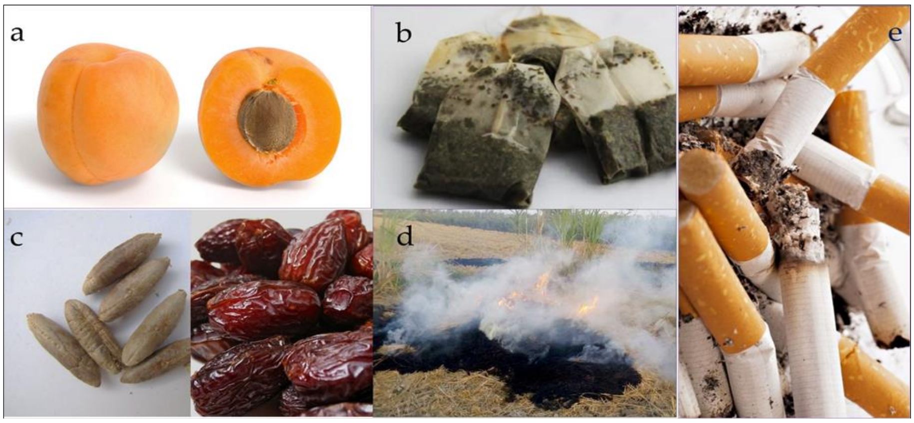

Fig 1: The plant waste materials: apricot kernels (a), Lipton-packets tea (b), date palm kernels (c), rice straw burnt (d) and cigarette filters (e).

2.3 Statistical analysis
The study was carried out using IBM SPSS Statistics for Windows Version 23.0 (IBM Corp., Armonk, NY, USA). The significance of the percentages of larval mortality was assessed using one-way analysis of variance (ANOVA), and Duncan's multiple range test was employed as the post hoc test. Data from lethal concentrations (LC50) were analyzed using probit theory. The significant levels were set at $p<0.05$.

3. Results
3.1 larvicidal activity of the plant waste extracts.
The larvicidal effects of plant waste extracts—apricot kernels, Lipton packet tea, date palm kernels, burnt rice straw, and cigarette filters were tested against 3rd larvae *Culex pipiens* indicating that mortalities were increased by increasing concentration and exposure time.

The mortality % of *Culex pipiens* after 24 h of exposure at 1500 and 2000 ppm of plant waste extracts; apricot kernels, Lipton-packets tea, date palm kernels, burnt rice straw, and cigarette filters were 59, 87, 71, 63, and 90% at 1500 ppm, and 79, 97, 90, 84, and 100% at 2000 ppm, 24 h, whereas the LC_{50} values (50%, median lethal concentration) of these extracts were = 1080.50, 582.42, 815.5, 976.21 and 528.14 ppm, respectively (Table 1&2).

Data showed that all plant waste extracts had larvicidal activity and Lipton packet tea (97%) was most effective followed by date palm kernels (90%), and burnt rice straw (84%) extracts, while apricot kernel (79%) extract were less effective. Also, the results showed that cigarette filter extract (100%) with LC_{50} value = 528.14, 415.91 ppm was more effective than Lipton packet tea (97, 100%) with LC_{50} value = 597.42, 470.91 ppm, at 2000 ppm, 24 and 48 h PT, respectively (Table 1&2 and Fig. 2).

3.1.1 Oviposition responses of female *Culex pipiens*
Data in Table 3 showed the effect of the five plant waste extracts on the oviposition, hatching, and non-hatching of female *Culex pipiens*. The rate of deposited eggs varied considerably according to apricot kernels, Lipton packet tea, date palm kernels, burnt rice straws, and cigarette filter extracts. The plant waste extracts were more attractive for oviposition, whereas the number of eggs laid was 818, 333, 350, 300, and 1355 eggs per 15 females at 1000 ppm concentration compared with untreated groups (1517, 1490, 1440, 1545, and 1580, respectively).

Data showed that cigarette filters and apricot kernel extracts were more attractive for female mosquitoes to lay eggs on. While both cigarette filters and date palm kernel extracts had significant effects on the decreasing hatching rate, the hatching rates reached 9.88% and 19%, respectively (Table 3).

Table 1: Efficacy of the plant waste extracts of apricot kernel, Lipton-packet tea, date palm kernel, rice straw, and cigarette filter on mortality of *Culex pipiens* after 24 and 48 exposures.

Time	Conc. (ppm)	Apricot kernel	Lipton packet tea	Date palm kernel	Burnt rice straw	Cigarette filters
24	0	$0{\pm}0^{gA}$	$0{\pm}0^{gA}$	$0{\pm}0^{gA}$	$0{\pm}0^{gA}$	$0{\pm}0^{gA}$
	125	$6{\pm}1.87^{fB}$	$11{\pm}1.87^{fA}$	$8{\pm}1.22^{fB}$	$6{\pm}1.87^{fB}$	$13{\pm}2.00^{fA}$
	250	$10{\pm}1.58^{eB}$	$20{\pm}1.58^{eA}$	$12{\pm}2.00^{eB}$	$10{\pm}1.58^{eB}$	$21{\pm}1.87^{eA}$
	500	$25{\pm}1.58^{dD}$	$33{\pm}3.39^{dB}$	$28{\pm}3.00^{dC}$	$27{\pm}2.00^{dCD}$	$36{\pm}2.45^{dA}$
	1000	$37{\pm}2.55^{cE}$	$57{\pm}4.64^{cB}$	$43{\pm}4.06^{cC}$	$40{\pm}3.54^{cD}$	$65{\pm}2.24^{cA}$
	1500	$59{\pm}3.67^{bE}$	$87{\pm}2.55^{bB}$	$71{\pm}5.10^{bC}$	$63{\pm}3.39^{bD}$	$90{\pm}3.54^{bA}$
	2000	$79{\pm}4.00^{aE}$	$97{\pm}2.00^{aB}$	$90{\pm}3.06^{aC}$	$84{\pm}2.45^{aD}$	$100{\pm}0.00^{aA}$
48	0	$0{\pm}0^{gA}$	$0{\pm}0^{gA}$	$0{\pm}0^{gA}$	$0{\pm}0^{gA}$	$0{\pm}0^{fA}$
	125	$10{\pm}3.54^{fB}$	$16{\pm}1.87^{fA}$	$12{\pm}2.55^{fB}$	$11{\pm}2.92^{fB}$	$18{\pm}1.22^{eA}$
	250	$14{\pm}2.92^{eC}$	$26{\pm}1.00^{eA}$	$17{\pm}2.55^{eB}$	$14{\pm}2.92^{eC}$	$28{\pm}2.00^{dA}$
	500	$29{\pm}2.92^{dD}$	$41{\pm}1.87^{dB}$	$34{\pm}1.87^{dC}$	$31{\pm}3.32^{dD}$	$44{\pm}3.67^{cA}$
	1000	$41{\pm}3.32^{cE}$	$67{\pm}5.39^{cB}$	$49{\pm}4.00^{cC}$	$45{\pm}3.16^{cD}$	$74{\pm}4.30^{bA}$
	1500	$63{\pm}4.64^{bE}$	$92{\pm}4.06^{bB}$	$75{\pm}4.74^{bC}$	$68{\pm}2.55^{bD}$	$98{\pm}2.00^{aA}$
	2000	$85{\pm}3.16^{aC}$	$100{\pm}0.00^{aA}$	$98{\pm}2.00^{aA}$	$90{\pm}2.74^{aB}$	$100{\pm}0.00^{aA}$

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter. A, B & C: There is no significant difference (P>0.05) between any two means, within the same row have the same superscript letter.

Table 2: Lethal time values of plant waste extracts against *Culex pipiens* after 24 and 48 exposures.

Time	Plant waste	LC_{50} (low-Up.)	LC_{90} (low-Up.)	LC_{95} (low-Up.)	Slope± SE	X^2 (sig.)
24	Apricot kernel	1080.50 (805.28-1618.94)	4981.72 (3715.34-12405.76)	7669.05 (5565.02-22756.08)	1.939±0.16	10.578 (0.031)
	Lipton packet tea	582.42 (360.41-936.65)	2140.81 (1754.45-5412.76)	3074.01 (2668.70-9163.61)	2.312±0.16	25.150 (0.000)
	Date palm kernel	815.5 (560.77-1211.64)	3643.39 (2917.88-9215.71)	5466.36 (4420.94-16456.71)	2.062±0.16	15.688 (0.003)
	Burnt rice straw	976.27 (707.82-1445.62)	4064.44 (3156.78-9577.10)	6089.68 (4680.22-16867.85)	2.069±0.17	12.673 (0.01)
	Cigarette filters	528.14 (309.45-823.54)	1789.19 (1447.38-4405.40)	2528.54 (2182.00-7279.41)	2.418±0.13	27.456 (0.000)
48	Apricot kernel	916.47 (609.87-1547.48)	4636.69 (3816.19-15893.38)	7341.74 (6172.98-31979.08)	1.820±0.15	16.444 (0.002)
	Lipton packet tea	470.91 (268.60-732.58)	1707.27 (1368.02-4289.48)	2459.62 (2105.59-7296.54)	2.291±0.15	26.331 (0.000)
	Date palm kernel	645.34	2757.77	4109.33	2.097±0.15	31.744 (0.000)

		(361.61-1240.05)	(2621.19-11772.26)	(4422.55-23153.56)		
	Burnt rice straw	807.06 (506.28-1378.93)	3712.49 (3203.30-12797.70)	5721.89 (5196.98-25025.61)	1.933±0.15	20.653 (0.000)
	Cigarette filters	415.91 (245.02-684.97)	1728.66 (1399.85-4754.41)	2554.43 (2206.93-8559.86)	2.212±0.16	22.719 (0.000)

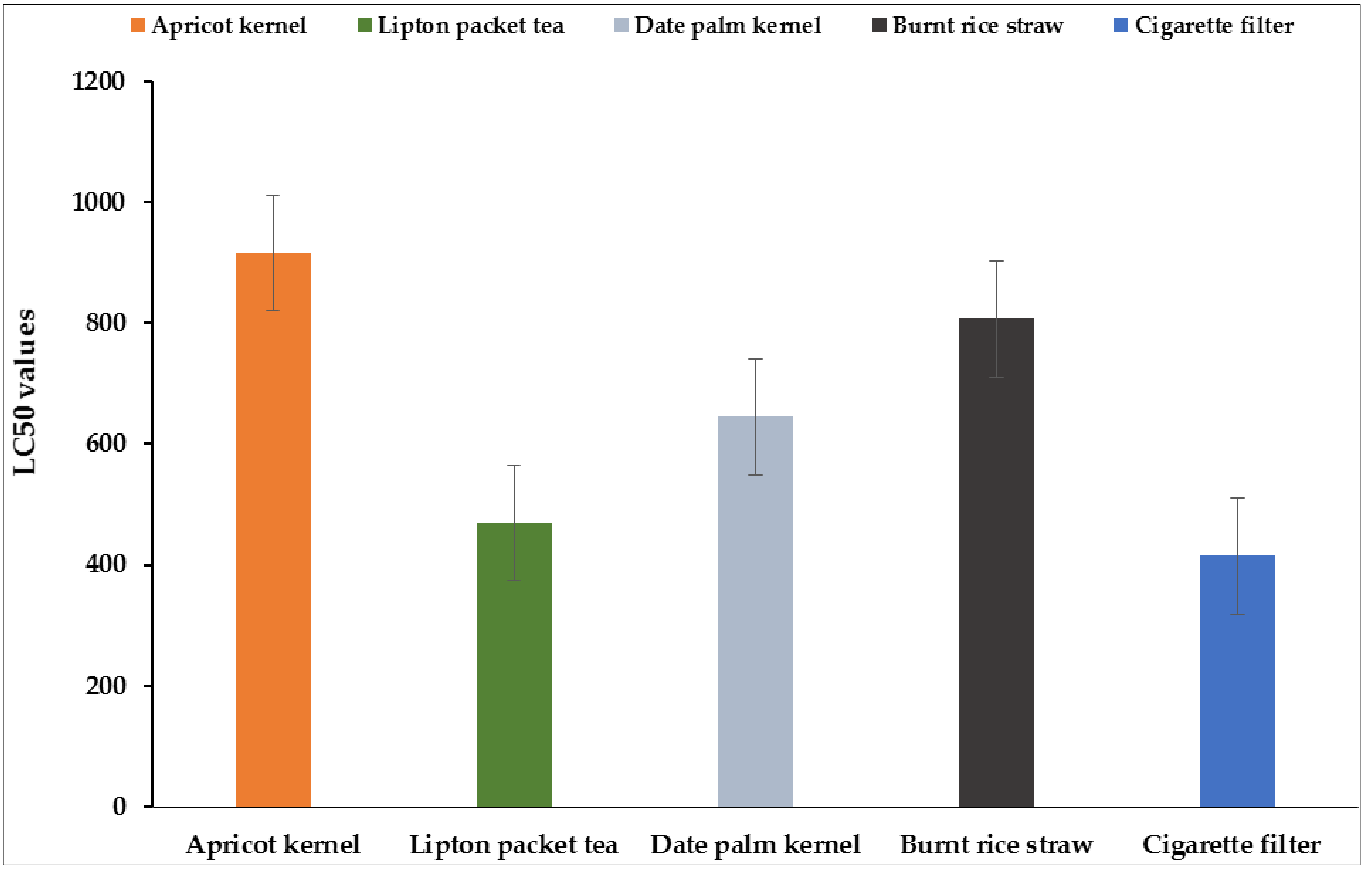

Fig 2: Larvicidal activity of plant waste materials; apricot kernels, Lipton packet tea, date palm kernels, burnt rice straw, and cigarette filters after 48 h of exposure.

Table 3: The impact of plant waste extracts on oviposition rate, egg hatching (%) and non-hatching eggs of *Culex pipiens*

Plant	Conc. (ppm)	Apricot kernel	Lipton packet tea	Date palm kernel	Burnt rice straw	Cigarette filters
Total egg	Control (0)	1517	1490	1440	1545	1580
	500	500	433	333	380	720
	1000	818	333	320	300	1355
No. of eggs hatched	Control (0)	1467	1432	1363	1477	1517
	500	427	333	243	282	333
	1000	370	152	207	182	277
No. of eggs non-hatched	Control (0)	50	58	77	68	63
	500	73	100	90	98	387
	1000	448	181	113	128	1078
Total hatching/ control (%)	Control (0)	100.00^{aA}	100.00^{aA}	100.00^{aA}	100.00^{aA}	100.00^{aA}
	500	29.09^{bA}	23.28^{bAB}	17.85^{bB}	19.07^{bB}	21.98^{bB}
	1000	25.23^{bA}	10.64^{cC}	15.16^{bBC}	12.30^{cBC}	18.24^{bB}
Total non-hatching/control (%)	Control (0)	0.00^{bA}	0.00^{cA}	0.00^{bA}	0.00^{cA}	0.00^{bA}
	500	70.91^{aB}	76.72^{bAB}	82.15^{aA}	80.93^{bA}	78.02^{aA}
	1000	74.77^{aC}	89.36^{aA}	84.84^{aAB}	87.70^{aAB}	81.76^{aB}
Hatching (%)	Control (0)	96.70^{aA}	96.09^{aA}	94.68^{aA}	95.58^{aA}	95.99^{aA}
	500	85.33^{aA}	76.92^{bA}	73.00^{bA}	74.12^{bA}	46.30^{bB}
	1000	45.21^{bA}	40.70^{cA}	24.58^{cA}	60.56^{bA}	12.42^{cB}
Non hatching (%)	Control (0)	3.30^{bA}	3.91^{cA}	5.32^{bA}	4.42^{bA}	4.01^{cA}
	500	14.67^{bB}	23.08^{bB}	27.00^{aB}	25.88^{aB}	53.70^{bA}
	1000	54.79^{aB}	54.30^{aB}	65.42^{aB}	39.44^{aB}	84.58^{aA}

a, b & c: There is no significant difference (P>0.05) between any two means for each attribute, within the same column have the same superscript letter. A, B & C: There is no significant difference (P>0.05) between any two means for each attribute, within the same row have the same superscript letter.

4. Discussion

Mosquito-borne diseases are among the most common public health problems in developing countries [25, 26]. It can be controlled by preventing mosquito bites by using repellents or hindering female mosquitoes from laying eggs, as well as by controlling mosquito larvae by safe methods, which leads to the death of many larvae and adult mosquitoes. Elimination of the larval stage is also beneficial because mosquito larvae cannot disperse or acquire human pathogens [27, 28].

The idea of evaluating some agricultural and industrial waste products of natural origin as larvicide agents against mosquitoes is a good and unconventional one since mosquito control requires new and innovative mechanisms that tends to nature. The primary criterion in selecting these compounds was the conversion of plant waste into useful materials, and they were easy and cheap to produce on a large scale.

Several previous studies were conducted mainly on botanical extracts of local plants in Egypt as well as around the world [28, 29-30] and their toxic effects on different types of insects [31]. In this study, the use of waste and its conversion into materials of value as an insecticide is evaluated. The tested extracts revealed differences in the types of plant and industrial waste extracts on the mortality rate of mosquito larvae, and these differences were attributed to the efficacy of the tested extracts and often to the main components of each.

Our findings demonstrated that cigarette filter extracts (100% mortality) were more effective than plant waste extracts at killing *Cx. pipiens* larvae, which had been exposed to all the plant waste extracts in our investigation after 24 and 48 h.

Several researchers recently evaluated cigarette butts, which are the unburned part of cigarette tobacco, along with a cigarette filter. The authors confirmed the toxicity of cigarette butts in killing mosquito larvae at a high rate, especially with increasing amounts of cigarette butts [32-35]. However, the effect of cigarette filters has not been studied; although they do not contain tobacco of any two flavors, burnt tobacco passes through them in addition to other components added to tobacco, which increases our belief that the cigarette filter contains toxic compounds that may reach or exceed the cigarette butts.

In a parallel study to our current work, Dieng *et al.* [36] recently studied the use of cigarette butts and their effect on changes in biological parameters from one generation to the next in mosquitoes. They examined the effects of three different concentrations of cigarette butt extract on mosquito egg-laying behaviors, egg hatchability, reproductive ability, longevity, and fertility. Finally, the mosquitoes that survived exposure to cigarette butts (CBs) waste had a short lifespan and altered life characteristics. As a result, the researchers confirmed the effectiveness of cigarette butt extract on mosquito larval mortality.

Previous literature showed that cigarette residues are huge wastes and amount to about 5.0 trillion [37] and that CBs contain toxic substances besides tobacco fibres, ash, and filtrate. US Department of Agriculture [38] referred to cigarettes as having about 10% by weight of additives known to increase toxicity [39].

Among the plant extracts, Lipton packet tea (97% mortality) and date palm kernel (90% mortality) were the most effective regarding lethal concentrations (LC_{50}) and had lower values compared to other plant extracts at 24 and 48 h post-treatment, respectively.

It has been proven through similar studies that tea leaves (*Camellia sinensis*) or green tea have an effective effect on killing mosquito larvae [28, 40-42]. The time of both larval and pupal development is significantly impacted when the young stages of the organism are exposed to *C. sinensis* leaf extract. These findings support a study by Dieng *et al.* [43] that indicated that dengue vectors treated to sublethal doses of *C. sinensis* leaf extracts had longer larval development times, lower pupation rates, and higher adult emergence rates. Because of its stimulating qualities, caffeine, a xanthine alkaloid, is a crucial component of green tea extract. Caffeine has been shown to interfere with the development of mosquito larvae [434 and may be effective against some pathogens [45].

We have hardly found studies on date palm kernels against mosquito larvae. Aqueous extracts of the fruit pulp, seed kernel, roots, bark, and leaves of *Balanites aegyptiaca* were tested for their ability to repel mosquito larvae of the *Cx. pipiens*. Larval mortality was highest in the aqueous root extract. In comparison to root and/or bark extracts, aqueous extracts of leaves, fruit pulp, and seed kernels revealed lower levels of larval mortality. All sections of *B. aegyptiaca* are thought to have larvicidal qualities that could be developed and utilised as organic pesticides to control mosquitoes [46].

Our data showed that the oviposition rate was higher in extracts of cigarette filters and apricot kernels, which were more attractive to female mosquitoes to lay eggs in. The reason for attracting female mosquitoes to lay eggs on the surface of extracted cigarette filters and apricot kernels is due to their light or dark brown color. Additionally, our data showed that palm kernel extracts and cigarette filters had a significant impact on the hatching rate. Where the percentage of hatching was low, which indicates the effect of these extracts on the death of mosquito egg embryos.

Our findings were consistent with those of Baeshen & Baz [47] and Zaitoun *et al.* [48], who investigated how *Acacia nilotica* extracts affected the ability to reduce egg hatchability and adult emergence. They discovered that acetone leaf extracts from *A. nilotica* were acutely toxic at 212.1 mg/L and chronically harmful at 144.2 mg/L.

Plant extracts contain a large, untapped stock of phytochemicals that could be widely used in mosquito control programs in place of synthetic insecticides. Several investigators have indicated the efficacy of phytochemicals against mosquito larvae based on their chemical nature and described the potential to kill mosquito larvae for a variety of plant-derived secondary substances [49].

Our findings concur with these findings. This study demonstrates that aqueous plant waste extracts of apricot kernels, Lipton packet tea, date palm kernels, burnt rice straw, and cigarette filters can be utilized as sustainable and eco-friendly mosquito repellents.

5. Conclusion

It is necessary to stop the devastating effects of the illnesses that mosquitoes spread on people and their pets. The use of natural products as ecologically benign pesticides is currently a major area of research due to the wide variety and high potency of phytochemical substances. Thus, this work provided evidence of the effectiveness of plant waste (apricot kernels, Lipton packet tea, date palm kernels, and burnt rice straws) and one industrial waste extract (cigarette filters) as an eco-friendly and cost-effective insecticide against mosquito larvae. Therefore, we recommend the conversion and use of many agricultural residues in the control of mosquitoes and insects of medical, veterinary, and economic importance. By transforming plant waste such as discarded rice straw into a useful, all-natural selective pesticide, the environmental issue posed by the rice straw can be resolved.

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