The Biocidal capacity of Datura (*Datura stramonium* L.) leaves and flowers and Cannabis (*Cannabis sativa* L.) seeds on some Mosquito's larvae (Diptera: Culicidae)

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Abstract
Plant-based alternative pesticides are safe, effective, eco-friendly and can be used instead of synthetic pesticides. The aim of this study was to evaluate the biocidal capacity of Datura stramonium leaves (DL) and flowers (DF) and Cannabis sativa seeds (CS) on Anopheles arabiensis, Culex quiquefasciatus and Aedes aegypti larvae. The selected plant parts were collected from the experimental farm, the University garden. The LC50s of A. arabinoses larvae towards the ethanol extract of each plant part were taken to estimate the single diagnostic dose for the survival and deformities tests. GC-MS analysis for the selected plant parts was investigated. The results show that DL ethanol extract detected 14 compounds of which butanol,3-methyl is the main component (79.76%) followed by toluene (6.14%). Phytol was also detected (3.9%). 14 compounds were detected from DF of which formic acid,3-methylbut-2-yl ester was the main component (82.22%) followed by dodecanoic acid, ethyl ester (3.3%) and toluene (2.86%). Also, from CS, Dronabinol (41.4%) was the main component, followed by Cannabinol (10.38%), Phytol (10.38%) and Caryophylene (2.07%). The susceptibility test of An. arabiensis larvae revealed the LC50's of 562.95 mg/L for DL, 424.41 mg/L for DF and 175.40 mg/L for CS.

It was noticed that 3-6% of An. arabiensis and 1-28% of Culex quiquefasciatus and 12-22% of Aedes aegypti larvae developed to pupae and some of them developed to adult stage after one week. In the survival test, more than 70% of the larvae were killed by a concentration supposed to kill 50% or less of the larvae. Some deformations were monitored on Anopheles, Culex and Aedes larvae including swelled body, separated alimentary canal. Field assessment should be run and the concept of LC50 should be re-written.

Keywords: Biocide, *Datura stramonium*, *Cannabis sativa*, mosquito's larvae.

Introduction
Cannabis sativa family Cannabaceae, has been cultivated throughout recorded history for many purposes: for example, the plant fibers can be converted into cloth. The plant has beneficial properties such as pain relief in addition to being an effective treatment for insomnia and an anxiolytic.

Globally, it is one of the most well-liked recreational drugs and it can be smoked, made into tea or mixed with food. It has been used as plant-based insecticide, miticide, or repellent, resulting in moderate efficacy of ethanol extract[7]. Beside cannabinoids, *Cannabis* chemical constituents include about 120 compounds responsible for its characteristic aroma. These are mainly volatile terpenes and sesquiterpenes (α-Pinene, Myrcene, Linalool, Limonene, Trans-β-ocimene, α-Terpinolene, Trans-caryophyllene, α-Humulene) which contribute to the characteristic aroma of *C. sativa.* There are more than 550 chemical compounds in cannabis with more than 100 phytocannabinoids being identified including tetrahydrocannabinol and cannabidiol[10].

Datura stramonium (Solanaceae) is a seasonal shrub invading the temperate regions of the world. It was known as a hallucinogen plant. Its phytochemical analysis shows that this plant contains tropane alkaloids (which is very toxic component)[3] and atropine (which is used in traditional medicine). The leaves are generally smoked[1] whereas in China, it was used during surgery in the form of anesthesia[15].

Biopesticides are usually associated with biological pest control and by the implication and manipulation of living organisms (e.g. plants, bacteria and other microbes). They became important components of Integrated Pest Management (IPM) programs[9]. They are usually applied as chemical pesticides. It achieved pest control in an eco-friendly manner. Biopesticides frequently target a very narrow species range. They rarely disturb many surrounding beneficial insects, vegetation and wildlife having various impact on the non-target organisms such as bees [14].

Synthetic insecticides were found to cause some ecological problems (e.g. water pollution), toxic action to non-target insects (e.g. pollinators) and insecticide-resistance cases across the world. Some plants possess some toxic components that can kills insect pest. For this reason, the biocidal capacity of the selected plant parts as biocidal agents against some mosquito's larvae was evaluated in this study.

Material and Methods
Materials: From the experimental farm, University of Gezira, Sudan, the selected plant parts which were: Datura leave (DL) and flowers (DF) and Cannabis seeds (CS), were collected, while mosquito's larvae (*Anopheles arabiensis, Culex quiquefasciatus and Aedes aegypti*) were identified and provided by the Insectary of the Blue Nile National Institute for Communicable Diseases (BNNICD), University of Gezira.

Preparation of extracts: Fresh DL, DF and CS were dried under shade, crushed to fine granules using an electrical blender and cool-extracted using 99% ethanol for 24 hours. The filtered extract was divided into two parts: one for the GC-MS analysis and the other was for the survival and biocidal tests.

GC-MS analysis: The prepared ethanol extracts of DL, DF and CS were analyzed using GC-MS device (QP2010 Ultra Shimadzu Europa GmbH, Library: NIST 11s.lib). The resulting data: peak number, name of the detected compounds, retention time (R.time), molecular weight, molecular formula and concentrations (% area) were presented in tables.

Biocidal tests: Following the instructions of WHO [16], 5 different concentrations of each of the ethanol extracts of DL, DF and CS were tested on *An. arabiensis* larvae. After 24 hours, LC50s were calculated. A single concentration of each extract (less than the LC50) was used against a population of 100 larvae of each mosquito species for 24 hours. The survived larvae were counted, transferred to plastic cups filled with fresh water and were monitored for one week. Control bathes were also designed. The morphological abnormalities of the dead and survived larvae, pupae and adults were photographed using a digital camera.

Statistical analysis: The obtained data were presented in table using suitable statistical tool for calculating LC50's. The morphological deformities monitored on the dead and survived mosquito's instars were presented as plates.

Results

GC-MS analysis: The ethanol extract of DL showed the presence of 14 compound (Figure 1), of which 1-Butanol,3-methyl (79.76%) was the main component followed by toluene, phytol, 3-azabicyclo[3.2.2] nonane, D-alanine and cystine (6.14%, 3.9%, 0.99%, 0.47% and 0.29% respectively). The ethanol extract of DF showed the presence of 14 compounds, the main compound of which was formic acid,3-methylbut-2-yl ester (82.22%) dodecanoic acid, ethyl ester, toluene, 3-methyl-oxiran-2-yl-methanol and carbamic acid, 2-(2-tolyoxycarbonylamino (3.3%, 2.86%, 2.1% and 2.04% respectively) (Figure 2).

Figure 3 showed the compounds detected form CS ethanol extract: dronabinol, cannabinol, 5-Androstene, 4-4-dimethyl, 6H-Dibenzo {b,d} pyran-1,8-diol, 6a, 7,8,9,10, Sulfurous acid, octadecyl 2-propyl ester, phytol, caryophyllene and caryophyllene oxide (41.46%, 10.38%, 4.59%, 3.97%, 3.15%, 2.86%, 2.07%, 1.93% respectively) and some other traces.

LC50 for *An. arabiensis* larvae: The susceptibility test for *An. arabiensis* larvae revealed LC50s for DL of 562.95 (mg/L), DF of 424.41 (mg/L) and CS of 175.40 (mg/L). From this values, the selected concentrations for the survival and deformities tests were: 402 mg/L for DL; 333 mg/L for DF and 160 mg/L for CS.

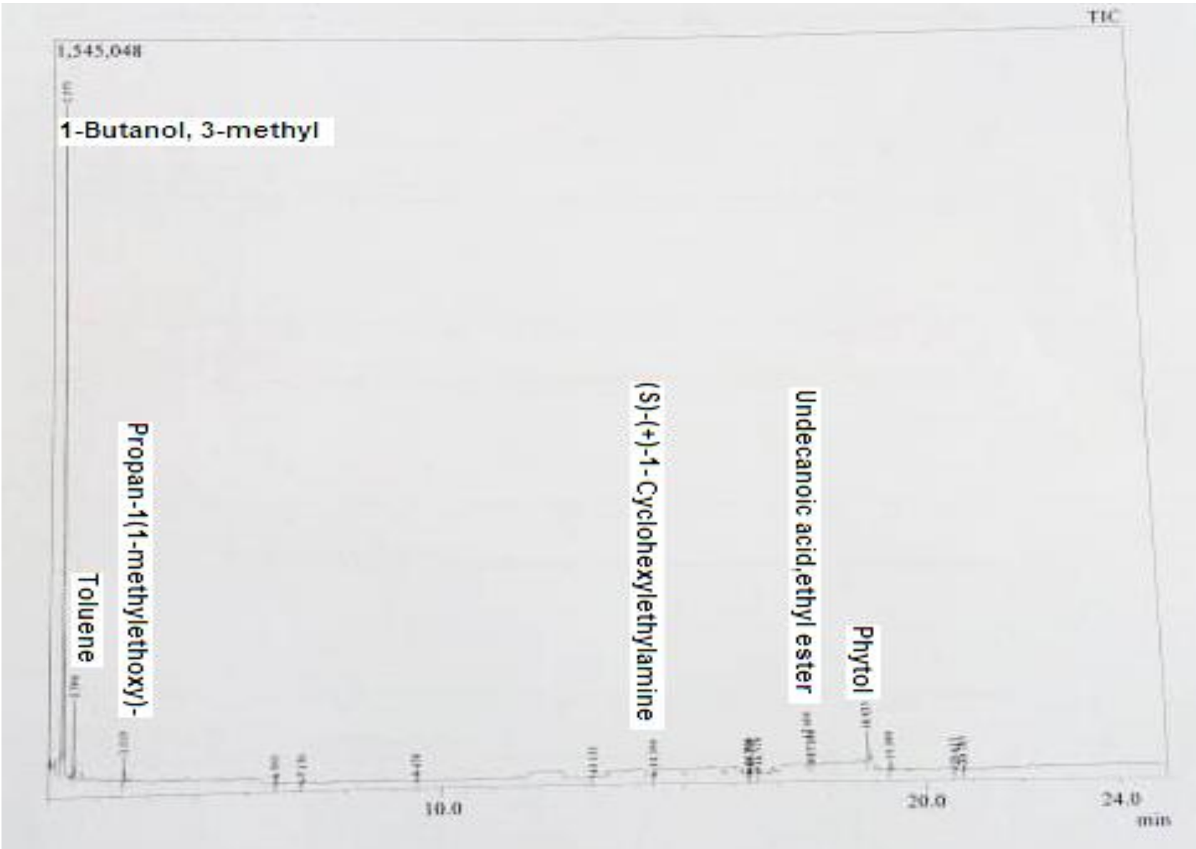

Figure 1: GC-MS chromatogram of the compounds detected from DL ethanol extract

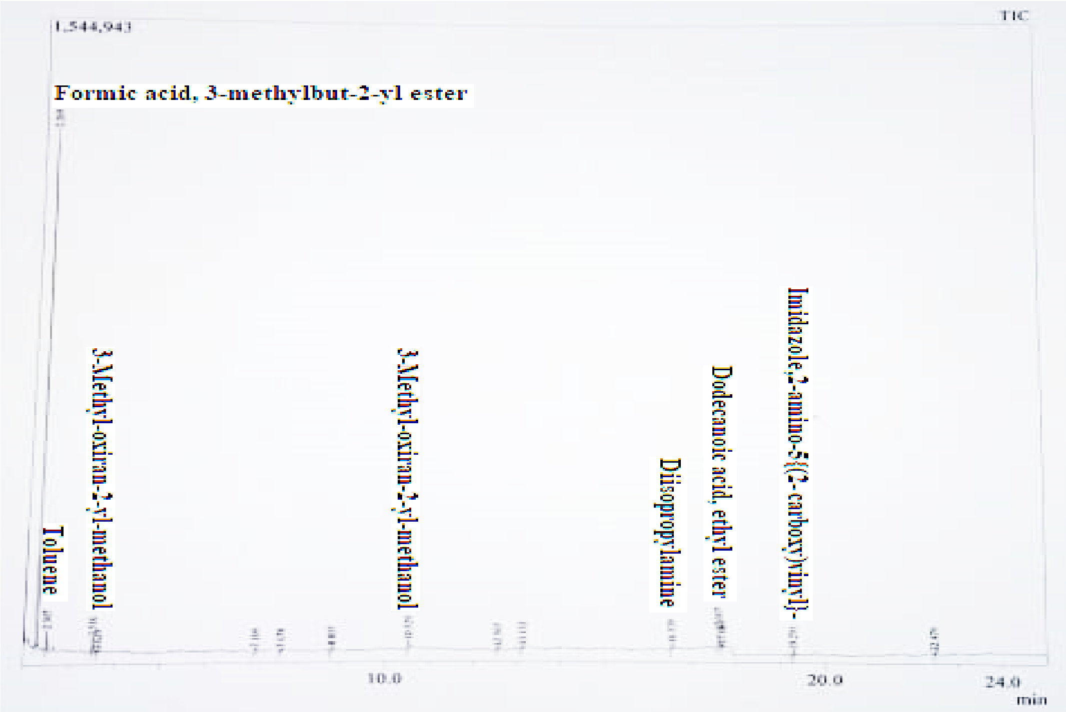

Figure 2: GC-MS chromatogram of the compounds detected from DF ethanol extract

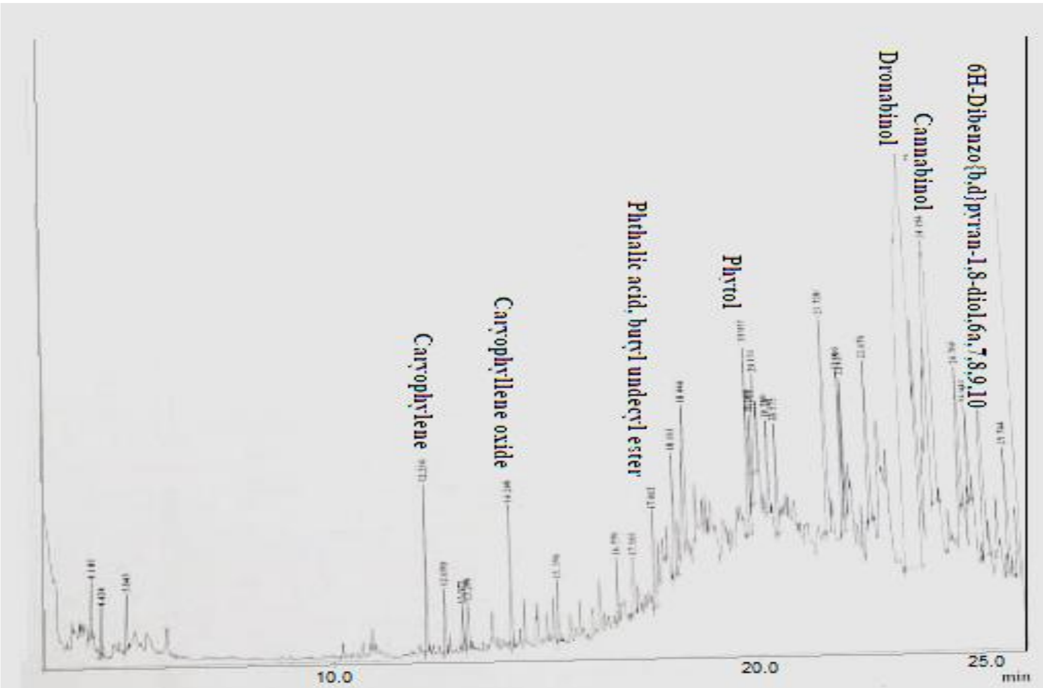

Figure 3: GC-MS chromatogram of the compounds detected from CS ethanol extract

The survival tests: Using the selected concentrations against the three mosquito's larval species for period of a week, the % survived, % developed and dead larvae are given in table 1. The control batches show that less than 10% of each of the tested larvae died after one week, while the selected concentrations produced more than 90% mortality in *An. arabiensis and C. quiquefasciatus* (except for DL) whereas the mortality of *Ae. Aegypti* ranged between 70-88%. It was noticed that about 3 – 6% of *An. arabiensis*, 1 - 28% of *C. quiquefasciatus and* 12 – 22% of *Ae. Aegypti* larvae were developed to pupae and further to adults after one week. Also, all *Anopheles* and *Culet* larvae developed or died, but 8% of *Aedes* larvae survived as larvae after one week at DL and CS tested concentrations.

The morphological deformities: The visual morphological changes noticed on *Anopheles* larvae caused by DF ethanol extract were: swelling, sclerotized mandibles, loose head connection, brightening of body color and disconnected alimentary canal, while some of the dead pupae showed black head and stretched body. Some of the developed adults emerged with short wings. The noticed deformities caused by DL extract were black-head dead larvae, some showed bright body color, swelled body, abnormal respiratory appendages, loose head connection and sclerotized mandibles while some pupae died and some emerged adults had swelled bodies. The noticed deformities caused by CS extract were not different than that of DL and DF (Plate 1).

Concerning *Culex* larvae, the noticed deformities caused by DF ethanol extract were brightening color, sclerotized mandibles and dark alimentary canal while some of the dead pupae showed black head, stretched body and incomplete emergence to adult. T

he deformities caused by DL were: swelled and brightening color and sclerotized mandibles of dead larvae, while some of the dead pupae showed stretched body and some dead adults.

Table 1
Survived, developed and dead mosquito larvae (%) after one week from exposed to the ethanol extract of *D. stramonium* leaves and flowers and *C. sativa* seeds

Product	Species	% Survived larvae	% Developed	% dead
Datura leaves (402 mg/L)	*Anopheles*	0	3	97
	Culex	0	28	72
	Aedes	8	17	75
Datura flowers (333 mg/L)	*Anopheles*	0	6	94
	Culex	0	1	99
	Aedes	0	12	88
Cannabis seeds (160 mg/L)	*Anopheles*	0	9	91
	Culex	0	10	90
	Aedes	8	22	70
Control	*Anopheles*	2	92	6
	Culex	1	92	7
	Aedes	2	95	3

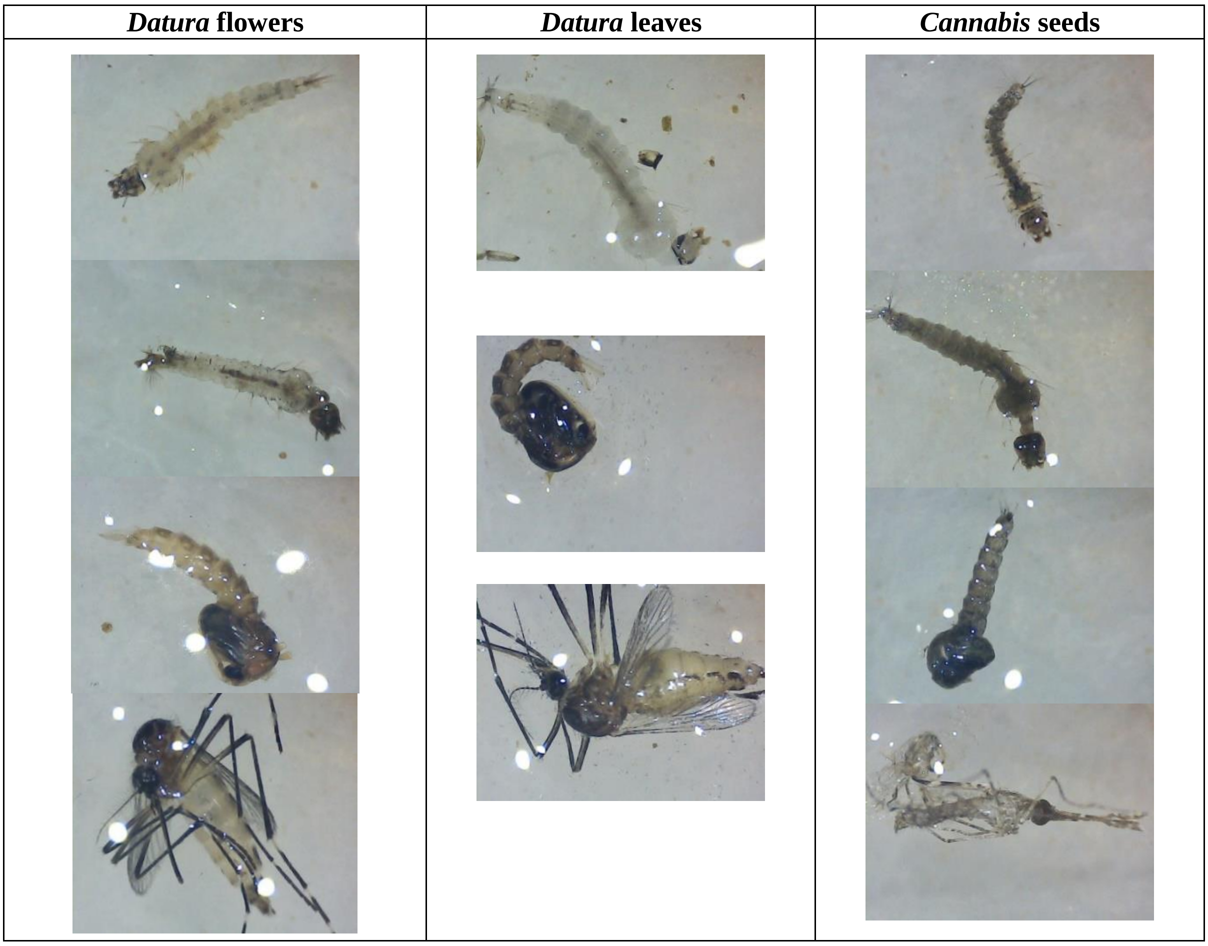

Plate 1: Deformities noticed on dead *Anopheles* larvae, pupae and emerged adults caused by *D. stramonium* leaves and flowers and *C. sativa* seeds

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The deformities caused by CS on some of the dead *Culex* larvae were thin body, sclerotized mandibles, brownish alimentary canal, while some of the dead pupae showed dark-head and incomplete emergence to adult (Plate 2).

Concerning *Aedes* larvae, some of the noticed deformities caused by DF ethanol extract were shed of antennae, brightening of body color, disconnected alimentary canal, died and stretched pupae in addition to short wings and swell-abdomen adults. The deformities caused by DL were the appearance of thin body, enlarged gut, swelling, black-head and stretched dead pupae and thin body adults. Some of the deformities caused by CS on the dead *Aedes* larvae were thin body, brownish alimentary canal, decapitate and some larvae unable to develop. Some of the dead pupae showed black-head, while some of the emerged adults were unable to flight (Plate 3).

Discussion

The GC-MS analysis for DL revealed the presence of the paint thinner (toluene), the diterpene (phytol) and the anti-plasmodium[11] agent (3-azabicyclo[3.2.2]nonane. The analysis also showed the presence of the non-proteinogenic amino acid (D-Alanine) and the sulfur containing amino acid (Cyctine). CS revealed the identification of Dronabinol (the main psychoactive component in marijuana) as the main component followed by Cannabinol (the mild psychoactive component found in trace amount in *Cannabis*), Phytol, Gamma-tocopherol, O-trifluoroacetyl (Vitamin E), Caryophylene (the monocyclic sesquiterpenes), Caryophyllene oxide (the cyclic sesquiterpenes), Pentacosanoic acid, methyl ester (a fatty acid) and other traces.

Similar study conducted in Pakistan at 2015 revealed the detection of delta-9-tetrahydro-cannabinol, cannabidiol and resorcinol, 2-p-mentha-1,8-dien-4-yl-5-pentyl in *C. indica* leaves, stems and seeds[4]. The susceptibility test of *An. arabiensis* larvae revealed that CS was more potent than DL and DF. Similar finding were stated by Mohammed [8] who found that CS was 4-folds more potent than DL and 3-folds more potent than DF against *Culex* larvae.

The ethanol extract of *Cannabis* leaves showed LC_{50} of 1000 mg/L (*An. stephensi*), 1400 (*Cx. quiquefasciatus*), 5000 (*Ae. aegypti*) within 24 hours[5], confirming the susceptibility of *Anopheles* larvae to be more than that of *Culex* and *Aedes* as was confirmed in this study against *Cannabis* leaves-ethanol extract.

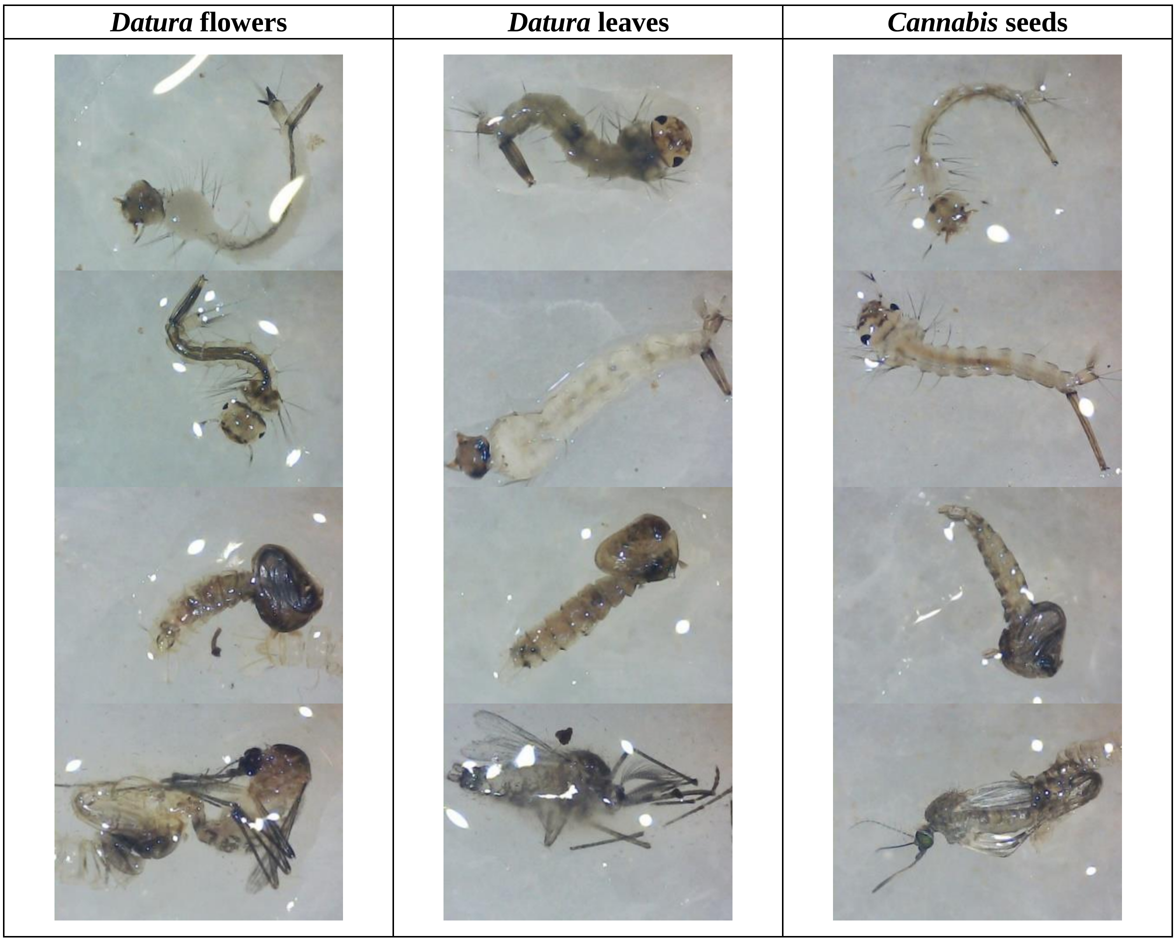

Plate 2: Some deformities on dead *Culex* larvae, pupae and emerged adults caused by *D. stramonium* leaves and flowers and *C. sativa* seeds

***Datura* flowers**	***Datura* leaves**	***Cannabis* seeds**

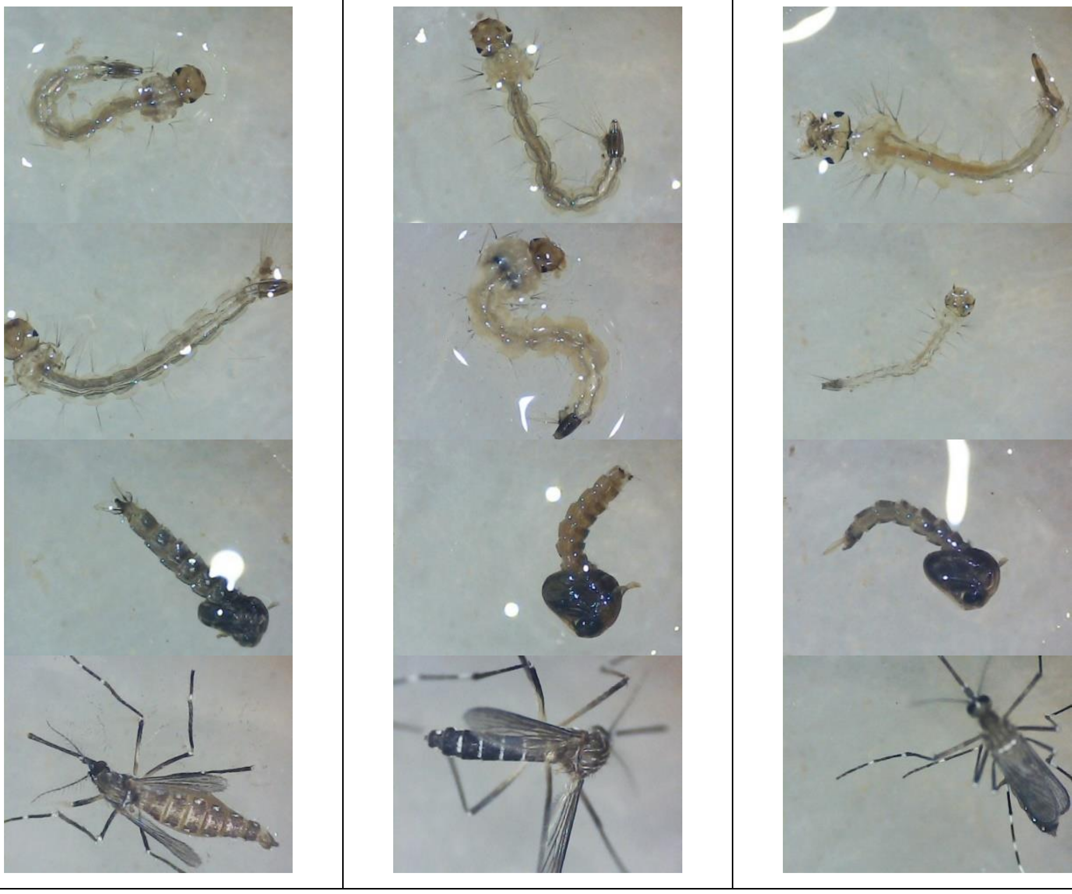

Plate 3: Some deformities on dead *Aedes* larvae, pupae and emerged adults caused by *D. stramonium* leaves and flowers and *C. sativa* seeds

The same finding was reported later by Swathi et al[12] who found that *Anopheles* was most susceptible to *Datura* leaves and flowers than *Culex* and *Aedes* larvae. The results were also confirmed by Mohamed[8]. The obtained results in this work show that less than 30% of all tested larvae were developed to pupil and adult stages after one week, also all larvae were developed to pupae after one week, except few *Ae. aegypti* larvae and this may be due to action of some or all the chemical compounds in the tested extracts on the moulting pathway.

The control batches showed that, less than 10% of the larvae of all species died after one week, while more than 90% of *An. arabinoses* died, whereas, 70-99% of *Ae. aegypti* and *C. quiquefasciatus* were killed after one week, although the single used dose was supposed to produce less or about 50% mortalities on each of the tested larvae. This finding suggested that the botanical insecticides have an extended impacts on the tested larvae, which is noticed also in their moulting disturbance. Also, the concept of LC50 should be re-written since the extended mortality after 24 hours is not considered on its common concept and calculations.

Ecdysone is a major insect molting hormone. It is of steroidal structure. Insect moulting hormones (ecdysone and ecdysteroids) act as moulting hormones of arthropods. Recent findings found a novel role of this hormone in regulating temporal gene transitions within neural stem cells of the fruit fly [13]. Ecdysone and other ecdysteroids also appear in many plants mostly as a protection agent (toxins or antifeedants) against herbivorous insects[2].

The use of insecticides in Saudi Arabia and some other countries has led to serious impacts on ecosystem. Botanists considered them to be as safe, eco-friendly, available and cheap alternative to synthetic insecticides. The selected plant parts in this study are examples of neglected plants that are flourished during the season when mosquitoes are breeds.

Many secondary metabolites from plant source are known to acquire insecticidal properties that can be used to kill or repel insects [17]. In this context, the capacities of the selected plant parts as biocides were evaluated and all of them are potential biocidal agents.

Conclusion

Although the used concentration is expected to produce mortalities equal or less than 50%, yet the experiment showed actually 70-99% mortalities of the larvae while some larvae were not developed to pupil stage, after one week, the used botanicals had an extended internal impact on moulting of the tested larvae.

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