Phytology

Evaluation of bio-insecticidal capacity of cannabis (*Cannabis sativa* L.) plants using GC-MS and phytochemical techniques

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Abstract

Plant-based pesticides are gaining attention as safe, effective, eco-friendly alternatives to synthetic pesticides. The aim of this study was to evaluate the bio-insecticidal capacity of Cannabis (*Cannabis sativa*) plants using GC-MS and phytochemical techniques and also mosquito's larvae as bioindicators. The phytochemical screening and the biological effect tests were run at Basic Sciences Laboratories, whereas GC-MS applications were run at the Central Laboratory, University of Gezira. The results showed that, *Cannabis* seeds contained flavonoids, alkaloids and steroids, and separated 5 spots through TLC. The GC-MS tests of *Cannabis* seeds detected Dronabinol (41.46%) as the main component, followed by Cannabinol (10.38%), Phytol (2.86%), Vitamin E (2.27%) and Caryophylene (2.07%). The ethanol extract of Cannabis seeds reflected LC50 of 218.1 mg/L, after 24. The more potent spot of Cannabis seeds was (3) against *Anopheles* larvae, but only spot (4) was more potent against *Culex* larvae and spot (5) against *Aedes* larvae. After one week of submission to Cannabis seed ethanol extract, the survived *Anopheles* larvae was 6.7%, while no survived *Culex* larvae, whereas 13.3% of *Aedes* larvae survived. Field assessment should be run to evaluate the sustainability of this products.

Keywords: Bio-insecticidal, Cannabis, GC-MS, Phytochemical Techniques

Introduction

Biopesticides are biological or biologically-derived agents that are usually applied in a manner similar to chemical pesticides, but achieve pest management in an environmentally friendly way. With all pest management products, effective control requires appropriate formulation and application (Matthews *et al*., 2014) [6]. Biopesticides rarely disturb the surrounding beneficial insects, vegetation and wildlife. It has lethal and nonlethal risks for non-target native pollinators (Tomé *et al*., 2015) [12].

Cannabis sativa (family Cannabaceae) is an annual plant. It has been cultivated throughout recorded history, used as a source of industrial fiber, food, religious and spiritual moods and medicine. Each part of the plant is harvested differently, depending on the purpose of its use (Greg, 2005) [2]. Globally, it can be smoked, made into tea. They also can be taken in herbal form, or unnaturally manufactured (Hazekamp *et al*., 2013) [3]. *C. indica*, a second species of *Cannabis* species, has been described. *C. indica* is well-suited for cultivation in temperate climates (MSNL Blog, 2017) [7]. This plants in the Indian Subcontinent are traditionally cultivated for the production of charas, a form of hashish (Fischedick *et al*., 2010) [1].

Both *sativa* (narrow-leaflet) and *indica* (wide-leaflet), are used as drug types. The *C. indica* has beneficial activity against pain, insomnia and an anxiolytic, while *C. sativa* gain common reports of a cerebral, creative and even, albeit rarely, comprising hallucinations (Seed Bank, 2012) [11]. Differences in the terpenoid content may account for some of these differences in effect (Karl, 2004) [4].

Beside cannabinoids, *Cannabis* chemical constituents include more than 100 compounds responsible for its characteristic activity (Novak *et al.*, 2001) [8]. Cannabis also produces numerous volatile sulfur compounds. These compounds are found in much lower concentrations than the major terpenes and sesquiterpenes. However, they contribute significantly to the pungent aroma of cannabis (Oswald *et al.*, 2021) [10].

Laws have been introduced in the United States, to permit the medical use of Cannabis (Office of National Drug Control Policy, 2015) [9].

The objective of this work was to study the phytochemical composition of *Cannabis sativa* seeds and to evaluate the larvicidal activity of its ethanol extract on three mosquitoes species.

Materials and Methods

Study Materials

The samples of Cannabis (*C. sativa*) seeds were brought from Singa, Sinnar State, Sudan. The larvae of mosquitoes (*Anopheles arabiensis*, *Culex quinquefasciatus* and *Aedes aegypti*) were brought from the insectary of the Blue Nile National Institute for Communicable Diseases (BNNICD),

University of Gezira.

Preparation of ethanol extracts

The selected plant parts were cleaned manually and then let to dry at room temperature away from direct sunlight, and then crushed to fine granules. Ethanol extract was prepared through cooled extract and was used to run the thin layer chromatography (TLC) and Gas Chromatography-Mass Spectroscopy (GC-MS) and to estimate the biocidal potentialities of this product using the mosquito larvae as bioindicators. Each of the spots that separated from the TLC test was scratched individually and dissolved in distilled water, filtered and used to test their larvicidal activities.

GC–MS analysis

The ethanol extract of cannabis seeds was analyzed using GCMS-QP2010 Ultra, Shimadzu Europa GmbH, device at the Central Laboratory, University of Gezira. The output involved the detected chemical named, their retention time, base peak, molecular weight, molecular formula and percentage area. The library used to identify compounds was NIST 11s.

Phytochemical screening tests

Phytochemical screening for the presence of the main classes (alkaloids, flavonoids, glycosides, saponnins, steroids and terpinoidsin and tannins) in cannabis seeds samples was done according to Khalifa and Kehail (2019) [5].

Thin layer Chromatography

The ethanol extract of cannabis seed was subjected to qualitative TLC following Khalifa and Kehail (2019) [5]. Each separated spot was used to test its individual biocidal potentiality. The mobile phase consists of acetone: hexane (80:20) mixture.

The biocidal potentiality

Following the instructions of WHO (2012) [13], the biocidal activity of cannabis seed was tested against *An. arabiensis, C. quinquefasciatus* and *Ae. aegypti* larvae. Three different tests were run: the first was to test the biocidal activity of the ethanol extract of the selected plant parts using only *C. quinquefasciatus* larvae, whereas in the second test the larvae of the three species were used to test the potentiality of each separated spots (from TLC test), and in both cases the test periods were 24 hours and based on three replicates. The third test was for survived larvae (using only one diagnostic concentration) and it continued for one week using the larvae of the three species. Control batch was designed for each test.

Statistical analysis

The data obtained were analyzed using suitable statistical tool. Probit analysis was used to calculate LC_{50} and LC_{95} for each product used.

Results and Discussion

The phytochemical screening

The phytochemical analysis of cannabis seeds, showed the detection of, flavonoids, alkaloids and steroids, while the others were not detected (Table, 1).

Table 1: Phytochemical analysis of *Cannabis sativa* seeds

Main class	Test result
Saponnins	-
Flavonoids	+
Tannins	-
Glycosides	-
Alkaloids	+
Steroids	+

(-) means absence; (+) means present of the main class

Thin layer chromatography test

The Thin layer chromatography (TLC) tests of cannabis seeds ethanol extract revealed the separation of only 5 active spots with different *Rf* values (Table, 2).

Table 2: TLC (*Rf* values) for *Cannabis sativa* seeds

Spot No.	*Rf* values
1	0.14
2	0.27
3	0.40
4	0.59
5	0.81

GC-MS tests

The GC-MS result of cannabis seeds (Table, 3) revealed the identification of Dronabinol (the main psychoactive component in marijuana; 41.46%) as the main component, followed by Cannabinol (the mild psychoactive component found in trace amount in cannabis; 10.38%), 5-Androstene,4,4-dimethyl (4.59%), 6H-Dibenzo {b,d}pyran-1,8-diol,6a,7,8,9,10 (3.97%), Sulfurous acid, octadecyl 2-propyl esrer (3.15%), then Phytol (the cyclic diterpine; 2.86%), n-Hexadecanoic acid (2.81%), 1H-4-Oxabenzo(f) cyclobut(cd)inden-8-ol (2.74%), E,E,Z-1,3,12-Nonadecatriene-5,14-diol (2.67%), Phthalic acid, butyl undecyl ester (2.63%), Z,Z-8,10-Hexadecadien-1-ol (2.55%), Gamma-tocophero, O-trifluoroacetyl- (Vitamin E; 2.27%), Caryophylene (the monocyclic sesquiterpenes; 2.07%), 2-methyltetracosane (2.0%), Caryophyllene oxide (the cyclic sesquiterpenes; 1.93%), Pentacosanoic acid, methyl ester (a fatty acid, 1.18%) and other traces.

Table 3: GC-MS detected compounds of cannabis seeds

Peak	Compound Name	Formula	Mol wt	R. time	Area %
1	Hexan, 2 nirto	$C_6H_{13}NO_2$	131	4.185	0.85
2	Hexane, 2,3,4-trimethyl-	C_9H_{20}	128	4.434	0.61
3	Cyclopentane, 1-acetyl-1,2,epoxy	$C_7H_{10}O_2$	126	5.045	0.80
4	Caryophylene	$C_{15}H_{24}$	204	12.216	2.07
5	Humulene	$C_{15}H_{24}$	204	12.650	0.95
6	Naphthalene, decahydro-4a-methyl-1-methyl	$C_{15}H_{24}$	204	13.073	0.59
7	Octadecane, 1-chloro-	$C_{18}H_{37}Cl$	288	13.206	0.82

8	Caryophyllene oxide	$C_{15}H_{24}O$	220	14.248	1.93
9	2-proponoic acid, pentadecyl ester	$C_{15}H_{34}O_2$	274	15.356	0.69
10	2-methyltetracosane	$C_{25}H_{52}$	352	16.779	0.72
11-12	Phthalic acid, butyl undecyl ester	$C_{23}H_{36}O_4$	376	17.163 17.662	1.18 1.45
13	1,2-benzenedicarboxylic acid, butyl 8-methylnonyl ester	$C_{22}H_{34}O_4$	362	18.161	1.74
14	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	18.444	2.81
15	Phytol	$C_{20}H_{40}O$	296	19.935	2.86
16	Pentacosanoic acid, methyl ester	$C_{26}H_{52}O_2$	396	20.008	1.18
17	Z,Z-8,10-Hexadecadien-1-ol	$C_{16}H_{30}O$	238	20.131	2.55
18-19	Sulfurous acid, octadecyl 2-propyl esrer	$C_{17}H_{36}O_3S$	320	20.389 20.575	1.44 1.71
20	5-Androstene,4,4-dimethyl	$C_{21}H_{34}$	286	21.728	4.59
21	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	294	22.066	2.67
22	7-Hexadecenal, (Z)-	$C_{16}H_{30}O$	238	22.125	1.58
23	1H-4-Oxabenzo(f)cyclobut(cd)inden-8-ol	$C_{21}H_{30}O_2$	314	22.676	2.74
24	Dronabinol	$C_{21}H_{30}O_2$	314	23.739	41.46
25	Cannabinol	$C_{21}H_{26}O_2$	310	24.194	10.38
26	Gamma-tocophero,O-trifluoroacetyl-	$C_{28}H_{48}O_2$	416	24.760	2.27
27	22-Tritetracontanone	$C_{43}H_{86}O$	619	24.941	1.41
28	6H-Dibenzo{b,d}pyran-1,8-diol,6a,7,8,9,10	$C_{21}H_{30}O_2$		25.249	3.97
29	2-methyltetracosane	$C_{25}H_{52}$	352	25.744	2.00

Biocidal tests

1. For the ethanol extracts on *Culex* larvae

The ethanol extract of Cannabis seeds (polar contents= 32.0%) was tested at concentrations of 160-560 mg/L on *Culex* larvae. The tested mortalities ranged between 35-90% after 24 hrs. The calculated LC_{50} was 218.1 mg/L (Table, 4).

Table 4: % mortality of *Culex* larvae on ethanol extract of Cannabis seeds

Concentration		Tested mortality (%)	Probit
mg/L	Log		
160	2.20	35	4.61
240	2.38	50	5.00
320	2.50	70	5.52
400	2.60	80	5.84
480	2.68	85	6.04
560	2.75	90	6.28
Probit analysis			
R^2		0.99	
Slope		3.13	
LC_{50} (mg/L)		218.10	
LC_{95} (mg/L)		728.82	

Control mortality= 0

2. For the separated spots by TLC

The larvicidal activity of each of the 5 separated spots of Cannabis seeds on *Anopheles* larvae produced mortality ranged between 15 to 35% (spot 3), while that of *Culex* larvae ranged between 40 to 70% (spot 4), whereas that of *Aedes* larvae ranged between 0 (spot 3 and 5) to 10% (spot 1) after 24 hrs (Table, 5).

Table 5: % mortality of mosquito's larvae on each of the (5) separated TLC-spots Cannabis seeds after 24 hours

Species	Spots				
	1	2	3	4	5
Anopheles	15	15	35	15	30
Culex	40	60	55	70	40
Aedes	10	5	0	5	0

3. For the survived mosquito's larvae

The ethanol extract of cannabis seeds (at concentration of 160 mg/L) was tested on *Anopheles*, *Culex* and *Aedes* larvae for one week to monitor the survived larvae (Table, 6). From the original number (60 individuals) of each mosquito's species, only 10 larvae (16.7%) of *Anopheles* were killed after 24 hours, while 5 larvae (8.3%) of *Culex* and one larva (1.7%) of *Aedes* were killed under the same concentration and period. After 48 hours the cumulative dead larvae increased to 20 (33.3%) in *Anopheles* with 38 (63.3%) survived. In *Culex* the dead larvae were 15 (25%) with 39 (65%) survived, while 5 (8.3%) larvae in *Aedes* were killed and 49 (81.7%) survived. After one week, the cumulative dead larvae reached 51 (85%) with 5 (8.3%) developed to pupae and 4 (6.7%) survived *Anopheles* larvae, while a total of 50 (83.3%) of the *Culex* larvae were died and 10 (16.7%) developed to the next instars, whereas, 30 (50%) of *Aedes* larvae were killed and 22 (36.7%) developed and no survived. The same product has an LC_{50} of 218.10 mg/L on *Culex* larvae after 24 hours (Table, 4). It was noticed that, *Anopheles* larvae were more susceptible to cannabis seeds more than *Culex* and *Aedes* larvae.

Table 6: Survived mosquito larvae on ethanol extract (at 160.0 mg/L) cannabis seeds during one week

Time	Species	Ref. No. Larvae	No. Dead larvae	No. Survived	Cumulative Developed	Cumulative dead
24 hrs	*Anopheles*	60	10	50	0	10
	Culex	60	5	53	2	5
	Aedes	60	1	56	3	1
48 hrs	*Anopheles*	50	10	38	2	20
	Culex	51	10	39	6	15
	Aedes	56	4	49	6	5
72 hrs	*Anopheles*	38	17	18	5	37
	Culex	39	17	19	9	32
	Aedes	49	7	38	10	12
One week	*Anopheles*	18	14	4	5	51
	Culex	19	18	0	10	50
	Aedes	38	18	8	22	30

Ref. No. larvae: the number of larvae survived at the end of the previous day

Chemical control is an effective strategy used extensively in daily life. However, the widespread use of synthetic insecticides has led to many negative consequences, resulting in increasing attention to natural products. Among bio-pesticides, botanical ones are experiencing a revival due to their eco-toxicological properties (Zoubiri and Baaliouamer, 2014). In this context, screening and evaluation of potentiality of cannabis seeds as bio-pesticides was the main concern of this study.

Phytochemical analysis in *C. sativa* indicated a high presence of steroids, alkaloids, flavonoids, Saponnins, tannins and phenols (Ahmed *et al*., 2019), whereas some of these phytochemicals were not detected in this study.

Cx. Quinquefasciatus larvae was submitted to methanol extract of Cannabis leaf, the LC50 (ppm) after 24 hours was (160.8) and after 48 hours was (71.1) (Maurya *et al*., 2008), whereas the ethanol extract showed LC_{50} of 1000 mg/L (*A. stephensi*), 1400 (*Cx. quinquefasciatus*), 5000 (*Ae. aegypti*) within 24 hours (Jalees *et al*., 1993), i.e. the susceptibility of *Anopheles* larvae was more than that of *Culex* and *Aedes*, and this finding was confirmed in this study against cannabis leaves-ethanol extract.

Conclusions

In summary, it can be concludedthat: Cannabis seeds contained flavonoids, alkaloids and steroids. cannabis seeds separated only 5 active spots. Cannabis seeds detected Dronabinol (41.46%) as the main component, followed by Cannabinol (10.38%), Phytol (2.86%), and Vitamin E (2.27%), Caryophylene (2.07%). The ethanol extract of Cannabis seeds reflected LC50 of 218.1 mg/L against Culex larvae, after 24. The more potent spot of Cannabis seeds was (3) against *Anopheles* larvae, and spot (4) against *Culex* larvae and spot (1) against *Aedes* larvae. The ethanol extract of cannabis seeds (at concentration of 160 mg/L), after one week, produced cumulative mortality of 85% but 8.3% developed to pupae and 6.7% survived on *Anopheles* larvae, while the mortality was 83.3% on *Culex* larvae but 16.7% developed to the next instars, whereas, the cumulative mortality was 50% of *Aedes* larvae and 36.7% developed and the rest survived. *Anopheles* larvae were susceptible to cannabis seeds more than *Culex* and *Aedes* larvae.

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