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RESEARCH ARTICLE

TERMINATING THE PROBLEM OF MOSQUITOES IN GAZA STRIP BY USING ALGAE.

Bassam F. S. Alzain.
Professor Doctor, College of Technology and Apllied Sciences, Al Quds Open University, Palestine.

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Abstract

In this study, the effect of Aqueous Extractors, Organic Solvent (Ethyl Ethanol) Hexane Alcohol) and the minor Hydroxylated represented by Adlumidine and Flavonoid of Chara sp. to words pupa of Aedes Albopticus. The results showed different effects for the extractors, which used in killing the Larvae. Hexane extractor has supremacy of LC_{50} and LC_{90} of mosquito Larvae reached 1000 and 3000 ppm respectively after 24 hours of treatment, and the rate of killing reached 65%, followed by Alcoholic Extractor with LC_{50} and LC_{90} reached 1000 and 4000 respectively, after 48 hours of treatment the rate of killing was 59.16%. Then Alkaloid Extractor with LC_{50} and LC_{90} reached 1200 and 6500 ppm after 48 hours of treatment, and the rate of killing was 53.33%. Finally, Flavonoid Extractor and Water Extractors are the least effective among the extractors used in killing ratio, which reached 35.05% and 5.16% respectively.

Introduction:-

The constant chemical processes used by chemical manufactured pesticides have formed a constant pressure on the insect community, and it has high and quick ability to develop resistance to these pesticides, this resistance will develop more quickly than the density pressure of chemical manufacturer of pesticides (Stipanovic, 1983). Which led to many new research directions in a agriculture pest management, known as third-generation insecticides (Ali and AbdellAziz,1986).

Since the Algae provides a suitable environment for the mosquitoes multiplication because it is considered as a key part in feeding mosquitoes (Zarzis and Amin, 1987),it could be used in controlling Mosquito Larvae and different types of Algae toxic to mosquito larvae were discovered since 1924. Since then, many researches indicated the possibility of using Algae in controlling through nutrition. Marten (1984) noticed that Kirchneriella irregularis has the ability to kill mosquito Larvae Aedes Albopticus when the later fed it, or by releasing its poison to the environment. Saario et. al (1994) observed that larvae of Aedes Aegypti were killed when fed strains of Algal Anabaena Circinalis and Oscillatoria Agardhi, because of its ability to release Microcystine poison. Some studies showed the effects of rough extractor isolated from algae toward Mosquito Larvae as (Watanabe et. al (1989) study founded that Methanolic extractor Lauvencia Nipponica has an effect to kill mosquito Larvae Culex Pipiens Palen. Many studies assessed the biological effectiveness of The Green Algae in the world including the study of Dhillon et. al (1982), Marten (1986), Berger& Schagerl (2004) and Ghazal et. al (2004). It includes Chara SP, which is species widespread all over the world and grows in fresh water or water half salty (brackish water) It also, has undesirable smell like the smell of onions, resultant from the presence of sulfur metabolites (Mayah and Hameem, 1991) It is one of Algae Charophyceae class, Charales rank (Imahor *&* wood, 1965).

Since the Palestinian environment is rich of many kinds of Greens Algae, this was chosen in an attempt to find natural alternatives for chemical manufacturers of pesticides used to fight Mosquito Larvae.

1) 

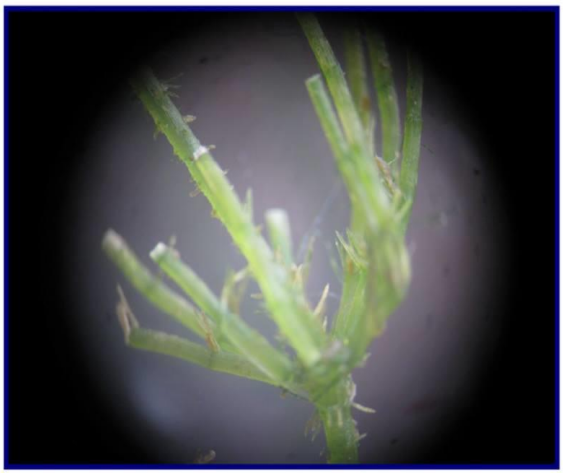
2) 

Image A:-The appearance figure of Alga Chara sp with the naked eye.

3) 

4) 

Image B:-Alga chara sp male and female organs .

5) 

6) 

Image C:-The microscopic shape of Alga Chara sp.

Materials and Methods of Work, Collecting and Breeding Mosquitoes (Aedes Albopticus):-

We followed the World Health Organization Method (WHO 1970) in mosquito breeding after collecting the Mosquito Larvae from a channel of waste water, drainage basins in the north of Gaza Strip, pools of stagnant water in agricultural areas, and Wadi Gaza. It was diagnosed by Prof. Bassam Al Zain professor of biology at Al - Quds Open University.

Collecting and Diagnosing Chara sp.

A sample of algae was collected from one of the swaps neighboring Wadi Gaza (Gaza Vale) agricultural areas in nylon bags and taken to the laboratory. The sample was washed with tap water several times to get rid of muds and little stuck impurities. Then the sample was dried on clean sheets in a shady place.

Chara sp diagnose included two kinds of subdivisions. The first one has unlimited growth and characterized by a length, containing nodules and internodes in addition to the existence of quasi-roots. The other is limited growth located near the nodules, normally short and in a circular motion around the node (panel 1-A). These branches is characterized by holding the female reproductive organs nucule and male reproductive organs (globule), that combines together in the breeding season to fertilized egg (zygote) with the black color, carrying construction called bud Scale Panel (1- B) Carts include thorns of different sizes and shapes and usually there is a verity distance between nods .The length of thorns has a significant impact on the classification to determine the type Panel (1- C).

Prepare Water Extractors

First, Dried algae sample was grinded. Then the water extractors were prepared by adding 10 grams of powder Algae to 200 ml of warm water distilled. Its temperature is not more than 50-60ْ C in a glass flask 500 ml, then keeping constant magnetic stirrer (IKA -COMBIMAG) for an hour. Then it was left down to settle for 30 minutes. Next, the solution was filtered by using part of Al-malmal cloth. The leachate was separated by a centrifuge (Kubote Model) Kc- 20 in speed 3000 cycles / minute for 10 minutes. The solution was filtered by filtering papers, type Whattma No1. After that, the leachate was collected; solvent was evaporated by Rotary evaporator at 45ْ C. Then the intensive leachate was put in a Petri pawl left to dry at room temperature. The dried material was weighed and preserved in glass bottles at a temperature 20ْ C until the use (Mansour, 1995).

Prepare Organic Solvent Extractors

Alcoholic extractors were prepared by adding 10 g of plant powder to 150 ml of Ethyl Alcohol 70% by using magnetic stirrer for 24 hours in a temperature 40ْ C. The resulting solution was filtrated by using Whattman paper filter, 1.NO. Then the solvent was evaporated by a rotary evaporator and at 45ْ C. After that, the intensive leachate was placed in a petri container and left to dry at room temperature. The dried substance was weighed and preserved in glass bottles at 20ْ C until the use (Harborne 1984).

Whereas, The Hexane Extractor was prepared by Soxhlet Extractor by adding 10 g of dry Algae in a paper container and using 200 ml of Solvent Hexane constantly for 24 hours. Then leachate and evaporation of the solvent were put in a Petri container and left to dry at room temperature, then it preserved in containers until the use at 20ْ C (Harborne 1984).

Chemical Detection Minor Metabolites

Detection of minor metabolites plant extractors were prepared by using reagents depositional the following:

1. To detect Alkaloids, use Mayer reagent, Diagendorff and Wagner reagent (Silva el al 1998).
2. To detect phenols, use Ferric Chlorid reagent (Shihata 1951) and Folin reagent (Harborne 1984).
3. To detect Flavonoids, use potassium hydroxide alcoholic KOH (Al- Khazraji)
4. To detect Tannins, use (Shilhata 1951) method.
5. To detect Saponins, use mercuric chloride HgCl 2 (1984 Harborne) and to detect frothiness (1998, Silva et. al).

Extracting Minor Metabolites

Extracting Alkaloids

According to Agarwel (1976) Alkaloids Metabolites was extracted by adding 25 g of dry plant powder in a paper container constantly (Thumble) by Soxhlet Extractor and mixed with 400 ml of Hexane Solvent for 24 hours, to isolate fat from the used plant sample. After leaving the sample, it was left to dry at room temperature to get rid of the remnants of the solvent, then the sample was mixed with 300 ml of Ethanol 96% in a glass beaker size 500 ml, using a magnetic stirrer for 24 hours at 45◌ C. Then the solution was filtered using evaporation papers type No.1 Whattman. The solvent was evaporated by a rotary evaporator at 45◌ C. The output separated was melted with distilled water and Acid 1% Acid Hydrochloride HCL until it reached PH of the mixture to 2, then it was left to settle for a while and it was filtered using filter papers for collecting leachate. 20 ml of Diethyl, Ether were added and separated by funnel to Acidic layer and other Atherah. Ammonia NH4OH was added to acid layer till the PH of the solution reached to 9. Then the solution was transferred to the separating funnel and added 20 ml of chloroform to it with well- shaken; and left to settle. Then the bottom layer of chloroform was separated and the process was repeated four times. The extracted Alkruforma was collected and evaporated by a rotary evaporator. Then it was left to dry at the room temperature. The dry material was weighed and preserved in glass containers at 20◌ C until the use. Alkaloids were detected by Dragendrohf reagent (Silva et al., 1998).

Extracting Flavonoids

Flavonoid metabolites were extracted using (Harborne, 1984) method if mixing 20 g of dry Algae powder with 300 ml of Acid solution of Hydrochloric HCL concentrated in 36% (2m) and they underwent extraction process by intensive inverter Reflex condenser using a water bath at 100◌ C for 40 minutes. After the solution had cool, it was filtered by using Whattman paper, NO 1. The leachate was transferred to a separating funnel and blend well with 50 ml of Ethyl. Two layers were produced; the upper layer of Ethyl Acetate was isolated. The process is repeated three times, the Ethyl Acetate Extractor was collected and dried, then it was saved in glass bottles at 20◌ C until the use. It was detected using a Detector Folin, Ferric Chlorid and Hydroxide Alcohol.

Study of the Influence of Prepared Extractors on the Death of the Fourth Instars Larvae of the Mosquito Aedes Albopticus

Six concentrations of Water Extractor, Alcoholic Extractor , Hexane Extractors, and minor Metabolites (alkaloids and flavonoids), The Chara Algae were prepared and used in the study which is concentrated in 30000, 10000, 5000, 1000, 500 and 100 ppm and Pupa Mosquitoes were isolated by using brush sketch of a thin edges according to Jubouri (1983) Larvae characterized with its big size and the head area is smaller than the dorsal area. Moreover, the eight parts of body are clear, the head holder pairs of distinctive feelers, Siphon has a long tubular shape. It was put in a petri dish contain distilled water or chlorine-free water.

(WHO 1970) method was followed in testing the toxicity of extractors prepared in mosquito larvae. Six plastic containers the size 150 ml and diameter 5 cm were prepared and added to three of them. 50 ml of prepared concentration while the other three repeaters were used to control with adding the same size of solvent (distilled water, ethyl alcohol 96% hexane) on them. Then ten Mosquito Larvae were transferred to each container (Random selection). Pots experiment were left at room temperature, and the dead Larvae were counted and recorded in each pots after 24 hours and 48 hours of treatment.

Statistical analysis:-

Killing percentage was corrected according to Abbot Formula, which mentioned in Shaban and Waterman (1993). These percentages were turned to values of the angle and subjected to statistical analysis according to the international complete experimental design , and in accordance to less significant rate method (D.S.L.R) and under probabilistic 05.0> P level and then LC_{50} and LC_{90} for death rates corrected using the computer EPA program.

Results:-

Results of Chemical Detection

The results of chemical disclosures made to the Chara Algae having both alkaloids and tannins and Alsabonyat and the absence of phenols and Flavonoids in aqueous extractor its presence in the Alcoholic Extractor. (Table 1)

Table 1:-Chemical statements for minor metabolites of Chara sp

METABOLITES Extractor	ALKALOIDS	phenols	flavonoids	tannins	Alsabonyat
AQUEOUS	+	-	-	+	+
ALCOHOLIC	+	+	+	+	+

(+) metabolites presence *(-) &* metabolites absence

The effect of the isolated extractors from Chara sp. in the percentage of killing larvae of mosquitoes Aedes Albopticus.

The results of the aqueous extractor, alcohol and hexane isolated from moss Kara extractor *:* Hexane has supremacy, kill ratio reaches 62.50% and 67.50% after 24 and 48 hours of treatment, respectively. Then followed by the alcoholic extractor a rate of kill ratio 53.33% and 65.00% during the durations of treatment and water extractor has less influence (table 2).

Table 2:-the effect of **aqueous**, alcoholic and hexane extractors on Chara sp in rates of the percentage of larvae of the mosquito Aedes Albopticus

Extractor type	time hour	Concentration (ppm)						rate of each killing time ratio	rate of each killing extractor ratio
		100	500	1000	5000	10000	30000		
aqueous	24	0	0	0	0	5	15	3.33	5.16
	48	0	0	0	7	10	25	7.00	
alcoholic	24	15	25	40	45	95	100	53.33	59.16
	48	15	30	60	90	95	100	65.00	
hexane	24	10	15	65	90	95	100	62.50	65.00
	48	15	30	75	90	95	100	67.50	
Killing rate to each concentration ratio		9.17	16.67	40.00	53.67	65.83	73.33	42.97	

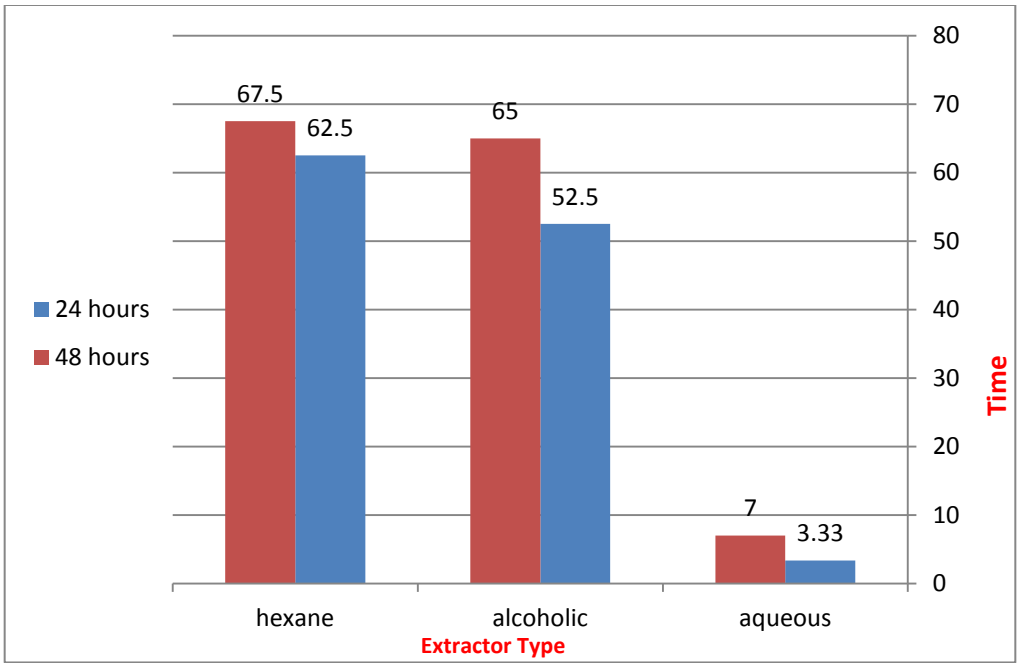
R.L.S.D influence extractor type .194 (0.05> P) R.L.S.D to the time effect 0.162 (0.05> P)
R.L.S.D to the effect of concentration 0.28 (0.05> P) R.L.S.D interference effect (Abstract type × concentrate) 0.486 (0.05> P)

R.L.S.D influence overlap (time × concentration) 0.41 (0.05> P) R.L.S.D the interference effect (Abstract type × time) 0.290 (0.05> P)

R.L.S.D interference effect (Abstract type x concentration × treatment duration) 0.711 (0.05> P)
The results of the statistical analysis of the time on (0.05> P) stated that the rates of killing increase with the increase of treatment duration therefore it is noticed that the highest kill ratio emerged after 48 hours of treatment. Statistical analysis of the concentration showed that there were significant differences between all concentrations and ratios of killing. Killing ratios increase with increasing concentration. It was higher than 30000 ppm concentration superior to the rest of the concentrations used.

The statistical overlaps between the extractor type and time stated high rates of killing when prolonging treatment for the aqueous and alcoholic extractors as killing rate increases of 3.33% and 53.33% respectively after 24 hours of treatment to 7.00% and 65.00% after 48 hours of treatment while there are no significant differences (0.05> P) during the time of treatment to hexane extractor. There are no significant differences recorded between the hexane alcoholic extractor at 48 hours (Fig. 1).

Figure 1:-shows the influence of the isolated extractor a type of moss Kara Chara sp.During the time of treatment on the larvae of the mosquito Aedes Albopticus



While statistical overlaps between the type of extractors and concentration showed, no significant differences at concentrations 30000 and 10000 ppm of alcohol and hexane extractor as rates of killings reached 100% and 95% respectively for both extractors in both concentrations .There are not registered significant differences for the last three concentrations (100, 500, and 1000 ppm) of aqueous extractors. Statistical overlaps between time and concentrations stated that the highest killing percentage recorded was of the concentration 30000 and 10000 ppm after 48 hours of treatment which did not record any significant differences in concentrations 30000 and 10000 ppm during the durations of treatment.

Through these results the mosquito larvae Aedes Albopticus reached less than less LC_{50} and LC_{90} of and 3000 ppm during the durations of treatment to hexane extractor followed by the alcoholic extractor and a concentration of 1000 and 4000 ppm within 48 hours of treatment. Table (3)

Table 3:-the value of killing concentrations LC_{50} and LC_{90} of the larvae of the mosquitoAedes Albopticus for the isolated extractors of the alga Chara sp.

Extractor type	Time	LC_{50}	LC_{90}
Aqueous	24	897 X 103	2905 X 103
	48	210 X 103	370 X 103
alcoholic	24	3200	15000
	48	1000	4000
Hexane	24	1000	3000
	48	1000	3000

While the results of the effect of alkaloids metabolites isolated flavonoids showed superiority of alkaloid to flavonoids extractors and at a rate of kill ratio stood at 49.81% and 54.80% after 24 and 48 hours of treatment (Table 4).

Table 4:-the effect of the active metabolites isolated from Chara sp on killing percentage of larvae of mosquitoes Aedes Albopticus.

Extractor type	time hour	Concentration (ppm)						rate of each killing time ratio	rate of each killing extractor ratio
		100	500	1000	5000	10000	30000		
alkaloids	24	3.10	3.8	10	90	95	97	49.81	52.30
	48	13.50	12.10	14	92	97	100	54.80	

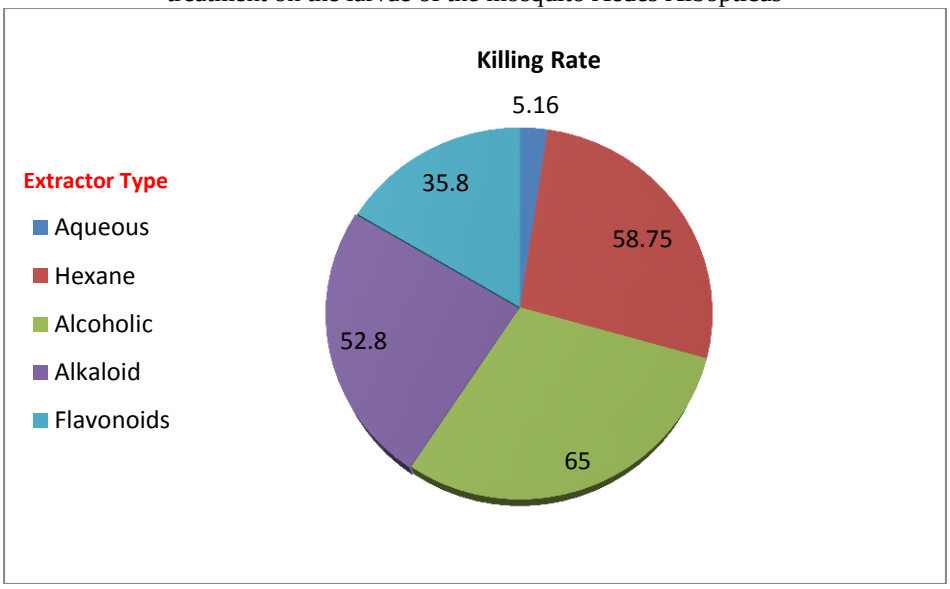
flavonoids	24	0	0	3	15	92	95	34.16	35.80
	48	0	1.00	6.7	25	94	98	37.45	
Killing rate to each concentration ratio		4.15	4.23	8.42	55.50	94.50	97.50	44.05	

R.L.S.D influence of extractor time.233 (0.05> P) R.L.S.D to influence of time 0.248 (0.05> P)

R.L.S.D to the influence of concentrations 0.387 (0.05> P) R.L.S.D interference influence (extractor type × concentration) 0.547 (0.05> P)

The results of statistical analysis showed, that on (0.05> P) concentration, there are significant differences between all the concentrations used except concentrations of 500 and 100 ppm. It is noticed that the killing percentage increase by increasing the concentrations, which used as the killing ratio increased from 55.50% to a concentration of 5000 ppm to 94.50% at a concentration 10000 ppm as shown by statistical analysis when prolonging the duration of treatment led to the increase killing rates significantly. The statistical interactions between time concentrations did not record any significant differences in the rates of killing by prolonging the treatment duration. We notice that the percentage of killing rate at 30000 ppm concentration reached 100% during the two durations of treatment and this is what was observed between the extractor and time as there are no any significant differences between them (Fig. 2) .

Figure 2:-shows the influence of alkaloid extractors and the isolated flavonoids of Charasp. during the time of treatment on the larvae of the mosquito Aedes Albopticus



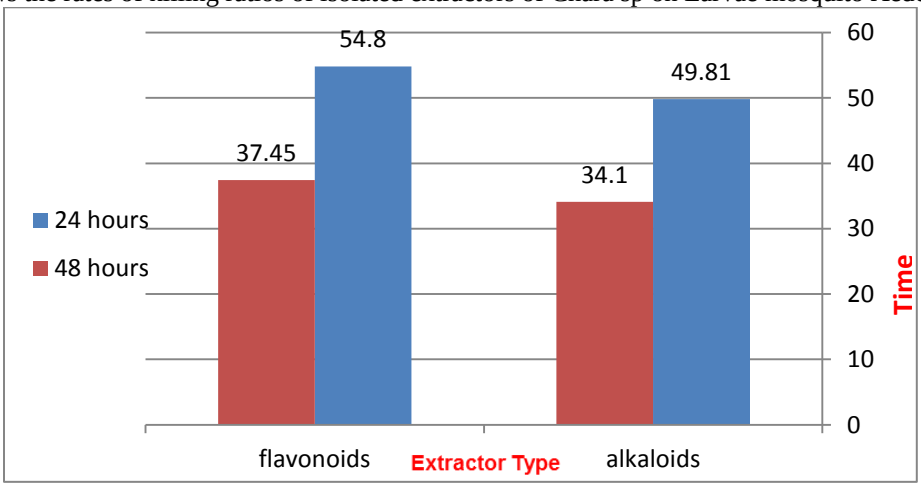
While statistical overlaps between the extractor type, user and concentration showed there is no significant difference between the alkaloid and flavonoid extractors at 30000 ppm and 10,000. These two concentrations have the superiority on the rest of the concentrations used. Thus, LC_{50} and LC_{90} 1200 and 6,500 values reached ppm to alkaloid extractor after 48 hours of treatment (Table 5).

Table 5:-showed killing concentration values for LC_{50} and LC_{90} of larvae of mosquitoes Aedes Albopticus to alkaloid and the raw isolated Flavonoids metabolites taken from Chara sp.

Extractor time	Time	LC_{50}	LC_{90}
alkaloids	24	2200	6500
	48	1200	6500
flavonoids	24	6500	15000
	48	6700	15000

Through these results , statistical overlap indicated that Hexane Extractor has superiority over all the extractors used and the rate of kill ratio reached 65.00%, followed by Alcoholic Extractor Alkaloid with a rate of killing ratio reached 59.16% and 52.30%, respectively, as shown in Figure 3.

Figure 3:-shows the rates of killing ratios of isolated extractors of Chara sp on Larvae mosquito Aedes Albopticus.



Discussion:-

The result of this study resembles Shaker study and others (2010) With different mosquito species, that shows Hexane Extractor and Gola extractor of Chara sp have supremacy on the other extractors (Figure 3). This is because of metabolites nature include these two extractor. (Marten,2007) shows alcoholic extractor of the alga Chara contraries includes 9 saturated fat and 5-unsaturated fats of 43.82% and 15.063% this extractor shows high effect as an anti-fungal when testing the effectiveness of the direction of 10 sick spun innate.

Zahir (2005) Hexane Extractor surpass on Alcoholic Extractors and Alkaloids and Flavonoids for a number of plants to kill mosquito Larvae Culex Pipiens Molestus. This is due to fats existing in Hexane Especially hexane solvent since the Hexane Solvent dissolves metabolites not polarity like fats. Also, most effective insecticides by touching are those materials that dissolve in solvents in Alkyotichael surface skin Epicuticle. Its function is water exclusion and Hydrophilic, but allows the entry of materials that dissolve in fat or lipophilic therefore works to bring its effect inside the body of the insect after entering (Zaid (1964). This is consistent with Dhillon et al. (1982) that Methanol Molecule extracted from Rhizoctoniumhierglphicum was the most toxic of gasoline and Alpetrolam extractor in killing Culiseta incidens, Aedes aegypti and Quinquefasciatus Cx that showed similar efficiency to (Juvenile hormone) and caused serious genetic alterations of new emergences and lateness in larval growth rates for 2-5 days. This was observed by (Choochate, et al.1999) The Hexane Extractors of plant Kaempferia galangal was more effective than Methanol Extractor on the same plant in killing of the fourth instar larvae of mosquitoes Culex quinquefasciatus.

The superiority shown by Alkaloid Extractor on Flavonoids Extractors is due to high efficiency of the Alkaloids is that these materials prevent vital metabolism in larvae (Jang et al. 2002) through the formation of complexes with proteins or with analyst enzymes. These metabolites containing inhibitor on the neurosecretory cells or influence directly on skin cells responsible for the production of enzymes responsible for tannin or influence Cuticular oxidation process (Jeyabalan& Murugan,1999). The lack of aqueous extractor may be due to not containing phenolic and Flavonoids Metabolites. As test detection of these metabolites shows (Table 1), Gross(2000) on the multiple of phenols of Myriophllum Spicatum have the ability to reduce Phytoplankton growth.

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