Dina Yousif Mohammed et al /J. Pharm. Sci. & Res. Vol. 10(3), 2018, 532-536

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

The effect of hot ethanolic algae extract of *Cladophora glumerata* on some biological aspect of *Callosobruchus maculates* (Fab)

Dina Yousif Mohammed, Sarah Ibrahim Mahmood ,Ahmed Sahi Dwaish
Sciences of College, University of Al Mustansiryia, Iraq, Baghdad

Abstract

The current study was carried out to determine the efficacy of algae extract as an alternative to the use of chemical pesticides , in this search used algae extract of Cladophora glumerata as a sample macro algae against some of the biological aspects of Callosobruchus maculates under laboratory conditions . Primary detection of active compounds showed the macro algae extracts containing Saponins ,Tannins ,Alkaloids , Flavonoids and phenols .Beside that, to identify the compounds which responsible of antifungal using Gas Chromatography-Mass Spectrometry.The algal extract that used at 2,4 and 8 mg / ml concentration showed higher efficiency to control of *Callosobruchus maculates*, tough their capacity diverges according to the extract concentrations, the laboratory results detect that the mortality rate of eggs were 60% ,68 %and 87% respectively, to decline to 0 % in the control treatment. There was an effect of on the adult, the mortality ratio were : 42% ,46% and 61% respectively .While it was 0 % in the control test. The results also showed that the effect of the concentration 8 mg/ml spores/ ml on the treated adults, reflected that effect in the rate of egg production the lowest rate of producing eggs was 18 when mated males treatment with the female treatment , and gave 29 eggs in the mated female treatment with the males non-treatment, also it was 44 eggs when mated males treatment with the female non-treatment, while the rate of eggs was 88 in the control treatment.

Key words: *Cladophora glumerata* **,** *Callosobruchus maculates*

INTRODUCTION:

The cowpea beetle *Callosobruchus maculates* (Fab) is one of the most important store pests, that infect different seeds of stored crops, the importance of this insect is due to its economic damage to the seeds as it feeds and develops its larvae inside the seed, as the eggshells appear attached to the seeds this leads to consuming a lot of weight and reduce its nutritional value [1,2] . Losses can occur up to 70 % of the weight of infected seeds without protection during nine months of storage [3]. The control of stored insect pests was largely dependent on chemical pesticides. But, the use of chemical control of storage pests is generally undesirable due to the risk of pesticide residues and consumer fear of contamination of their crops from the poisons of those pesticides [4]. Therefore, the researchers have found another safe alternative method to control store insects such as the use of substances that have a negative impact on the pests and safe for the human and animal and does not cause a significant imbalance in the ecosystem such as: plant extracts and algal extract. Due to random used of chemical compound to vanish the insect, different studies try to found new source as anti-insect agents with main condition their natural origin and low chance of insect developing and resistance. Beside that , low adverse effect on physiological processes of plants and less environmental hazards compared to their synthetic alternatives, being algae products are easily converted into a common organic material (eco-friend) [5,6] *Cladophora* is green macro-algae a branching, benthic, attached on rocks and submersed wood exposed to direct light and in extreme cases will grow on plants also. Appear as filamentous that forms a net like structure. Widespread in marine and freshwater habitats [7,8] .This alga doesn't appear to be slimy. The threads are tiny and very hard Usually it tends to stay in one spot, which makes it easy to remove [9,10].

As the importance of the insect and its economic damage to legumes in general and the fact that pesticides affect the human and environment, the objective of the current research to evaluate the effectiveness of the algae extract of *Cladophora glumerata* as an alternative to the use of chemical pesticides.

MATERIALS AND METHODS

Collection *C. maculatus*

C.maculatus was getting it from the Faculty of Agriculture / University of Baghdad, the culture was prepared by putting pairs of insects (Male and female) on the bean at 14 cm x 10 cm plastic containers, the culture was wrapped in a transparent cloth fixed on the top of the container with a rubber band to prohibit the insects from escaping it, incubated at 27 ± 2 ° C and 65 ± 5 % relative humidity until the treatment time.

Collection and preparation *C. glomerata*:

During autumn 2017 ,the macro algae were collected manually from Al Rashidiya region in north of Baghdad city ,that can be found at longitude 44°20'15.62"E and latitude 33°36'12.29"N. The sampling began at about 9A.M.and finishing at 12P.M Samples of *C. glomeratawere*, then transported to the laboratory after was stored in plastic bags. The specimen was cleaned from sand, shells, dirt etc... The identification of the macro-algae was depending on [11] and [12]. The specimen was dried at 50°C in an oven and then grounded to powder by the blender.

Preparation of extract:

Soxhelet extraction was used to prepared alcoholic extract according to [13,14]. Dried powder form of macro-algae material extracted by using ethyl alcohol. The concentrated active constituents from macro-algae were kept in sterilized test tubes stored in refrigerator till further use. The traces of methanol were removed by keeping the tubes at 50˚c for 1 hr

Detection the active compounds :

From adopting standard protocols [24] to detected the presence or absent of active compounds in macro algae .

Gas Chromatography-mass Spectrometry:

For GC-MS analysis, a high-temperature column (Inert cap 1MS ; 30 m x 0.25 mm id x 0.25µm film thickness) was purchased from Agilent Technologies (SHIMADZU – Japan). By employing a high- temperature column, we eliminated the need for derivatization of each sample. The injector and detector temperatures were set at 280°C while the initial column temperature was set at 100°C. A 5µl sample volume was injected in to the column and ran using split (1:10) mode After 1 min, the oven temperature was raised to 225°C at a ramp rate of 12.5°C/min (hold time 4 min) . The oven temperature was then raised to 300°C at a ramp rate of 7.5°C/min(hold time 5 min) . The helium carrier gas was programmed to maintain a constant

flow rate of 17.5 ml/min and the mass spectra were acquired and processed using both Agilent GC-Mass.Solution (SHIMADZU – Japan) and Postrun software. The compounds were identified by comparison of their mass with NIST library search and authentic standards.

Effect of extract algae C. *glumerata* on 24 hours old Eggs of C. *maculates*

20 eggs, divided into five replicates were used in the experiment; one egg on each seed put in petri dishes and treatment by direct spraying of the algae extract at a distance of 15 cm with three concentrations (2, 4, 8 mg\ml) in addition control test. Dishes were moved to incubate at 27 ± 2°C and 65±5% relative humidity; mortality of egg ratio and hatching time were recorded.

Effect of the extract algae C. *glumerata* on adult mating and egg production

The adult was obtained after the follow-up pupa, and developing into adult, differentiate between the males and females depending on the size of the female largest size of the male with two dark lines at the end of the female abdomen. , adults (male and female) were put in a petri dish Treatment by direct spraying of the algae extract a distance of 15 cm at 8 mg/ml concentration in addition control treatment.

The following mating was carried out:

A. Male treated and female treated.
B. Male non-treated and female treated.
C. Males treated and non- treated females
D. Males and females non-treatment (control treatment).

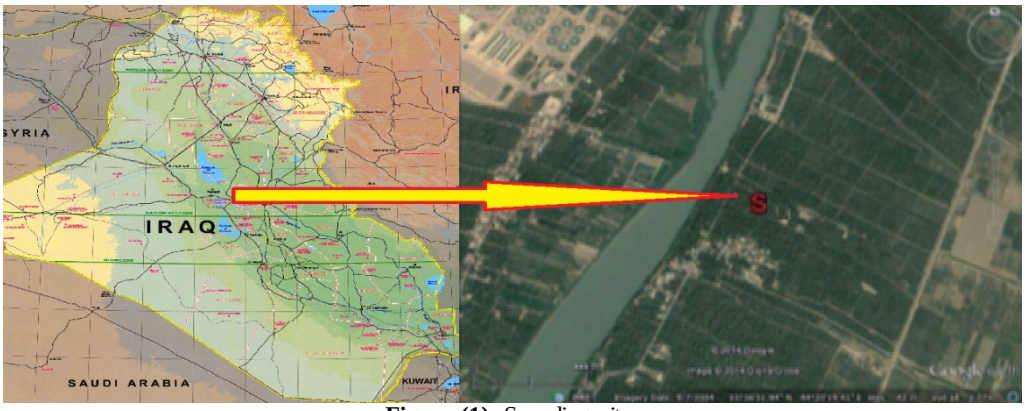

Figure (1): Sampling site.
S: Al-Rashidiya region (located on longitude 33°36'01.94"N and latitude 44°20'19.41"E).

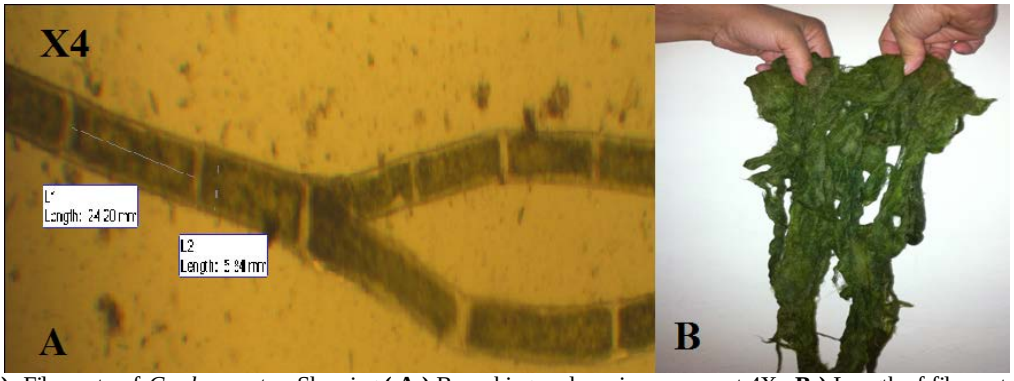

Figure (2): Filaments of *C. glomerata* . Showing **(A)** Branching under microscope at 4X **. B)** Length of filament in nature.

RESULTS & DISCUSSION

Morphological Structure of *Cladophora glomerata*:

The macro algae *C. glomerata* belong to phylum Chlorophyta ,that appearing green or light green, filamentous in form, attached on rock of shallow rivers. Microscopically, thalli are composed of joined cylindrical cells, with lengths of 6 – 20 µm and widths of 4 - 10 µm and with dichotomously branching filaments. (Fig. 2) .

Evaluation of Phyto-active compounds:

The current study analysis phytochemical hot ethanolic of *C. glomerata* extracts which revealed that Saponins ,Tannins ,Alkaloids , Flavonoids and phenols are generally present. While, other metabolites such as Glycosides and Terpenoid were absent in the extract table (1) .This result is agree with other findings [15,16,17,18,19]. The total yield of hot ethanolic *C. glomerata* was determined as (15) % from all algae mass that used.

Table (1): The active compounds in extract of *C. glomerata*

Active compounds	*Hot ethanolic extract of C. glomerata*
Alkaloids	+
Glycosides	-
Tannins	+
Terpenoid	-
Flavonoids	+
Phenols	+
Saponins	+

GC-MS Analysis:

Ethanol extract of green seaweed *C. glomerata*were dissolved in hexane and subjected to GC-MS to analyze the chemical constituents. Characteristic Gas Chromatograph-Mass Spectrometry analysis of hydrocarbons has been summarized. In the active fraction, Hexadecane-tetra methyl was found to be a

major compound (42.03%) followed by Pentadecanone (12.67%) and Salicylic acid(12.5%). Also followed by Octadecane -8-Methyl (11.2%) and Hexadecaden (6.7%) (Table2) and (Figure 3).

Table (2): The major identified compounds of hot crud metabolic extract of *C. glomerata* by using GC-Mass spectrophotometer

Rt*(min)	Compounds	Rt Compound Area %
11.32	Salicylic acid	12.5
12.72	Pentadecanone	12.67
13.76	Hexadecane	4.6
14.11	Hexadecane-tetra methyl	42.03
16.77	Tetradecane -8-Methyl	3.02
17.07	Octadecane -8-Methyl	11.2
21.8	Hexadecane	6.7
24.07	Nonadecane	6.1

* Rt= Rotation time

As see in table (2) 8-major compounds found in a hot ethanolic crud extract of *C. glomerata*,, these were: Salicylic acid is a monohydroxybenzoic acid, a type of phenolic acid and a beta hydroxy acid. This colorless crystalline organic acid is widely used in organic synthesis and functions as a plant hormone. It is derived from the metabolism of salicin which found in area in 12.5% from the analysis by GCMass.

Hexadecane -tetra methyl this compound belong to the acyclic diterpenes. These are diterpenes (compounds made of four consecutive isoprene units) that do not contain a cycle, that had antimicrobial activity, and it's found in area 42.03% from the analysis by GC-Mass . Also, Octadecane -8- Methyl which belong to hydrocarbons class which had several bioactivity such as Lubricant, Transformer oil, Anti-corrosion agent and Pheromones. Also, in table (2). Nonadecane (6.1%) is an alkane hydrocarbon .The alkane hydrocarbon is the generic name for the group of aliphatic hydrocarbons Cn-H2n+2 ,which represented reactive groups. Similar group of hydrocarbons Tetradecane, Octadecane and Hexadecane have been reported as common major volatile components in the crud extract of macro-algae *C. glomerataand* this results agreed with other studied such as [25,26].

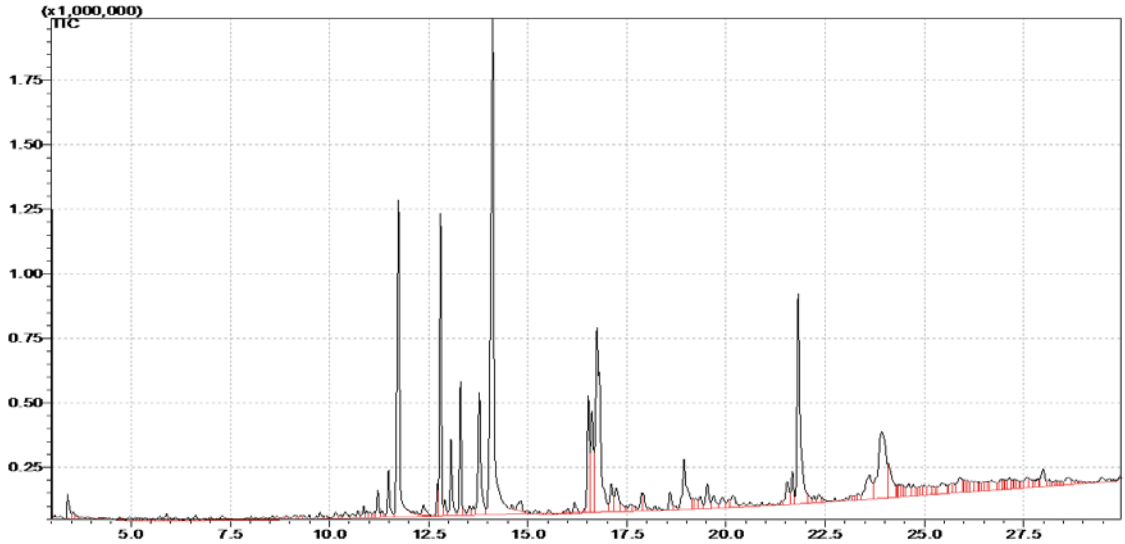

Figure (3): The chromato of GC-Mass spectrophotometery showed that hot extract of *C. glomerata* was a mixture of at least 8 compounds.

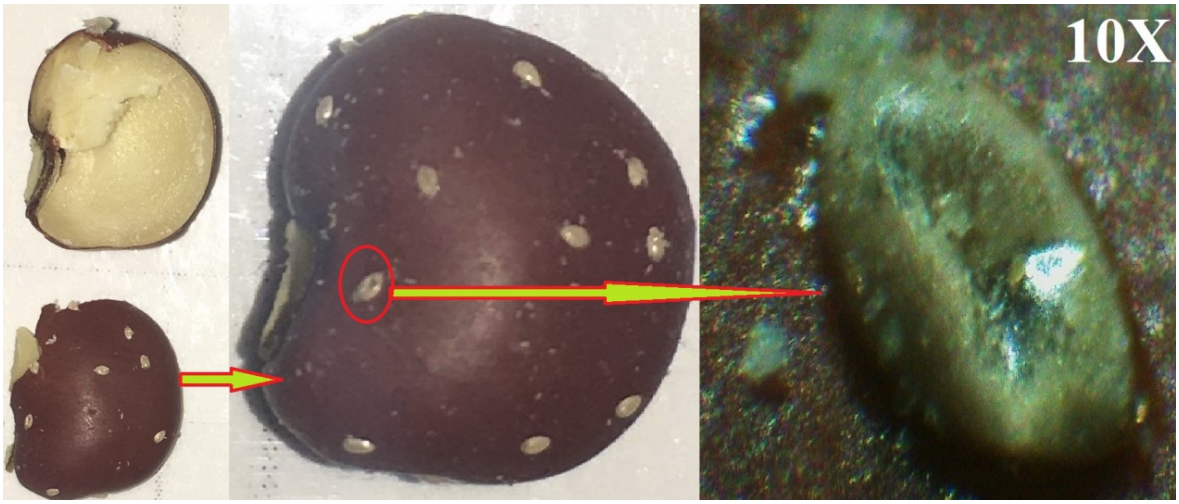

Figure (4): Eggs of C. *maculates* after treatment with extract algae C. *glumerata* at 8mg/ml

Effect of extract algae C. *glumerata* on 24 hours old Eggs of C. *maculates*

The results in Table 3 showed a significant impact of algae extracts on the eggs mortality. The highest percentage mortality of eggs was 87% at 8 mg/ml concentration and 4 mg/ml gave 68%, while 2 mg/ml gave 60% which to decline to 0% in the control treatment. As for the hatching period, there were not found significant differences in all treatments.

Table (3) Effect of extract algae C. *glumerata* on Eggs of C. *maculates*

Insect	Concentrations	Mortality of egg rates %	Hatching period (days)
C. maculates	8 mg/ml	87	6.7
	4 mg/ml	68	5.0
	2 mg/ml	60	6.0
	Control	0	6.0
LSD value		9.526 *	0.731 *
* (P<0.05).			

Effect of the extract algae C. *glumerata* on adult mating and egg production

Data presented Table 4 showed a significant impact of the algae extract on the adult at 8 mg\ml, the mortality ratio was 61% , 46% , 42% at 8, 4 , 2 mg\ml concentrations respectively While it was 0 in the control test.

Table(4) Effect of extract algae C. *glumerata* on adult of C. *maculates*

Insect	Concentrations	Mortality rates %
C. *maculates*	8 mg/ml	61
	4 mg/ml	46
	2 mg/ml	42
	Control	0
LSD value		8.026 *
* (P<0.05).		

The results in table 5 showed that effectiveness of the concentration 8 mg/ ml in the treated adults, reflected that effect in the rate of egg production, the lowest rate of producing eggs when mated females treatment with males treatment, gave the rate of egg production the lowest rate of producing eggs was 18 in the A treatment and gave 29 eggs in the B treatment, also it was 44 eggs when C treatment, while the rate of eggs was 88 in the control test figure (4).

Table (5): Effect of extract algae C. *glumerata* on adult mating and egg production

Insect	Mating	Rate of egg	Hatching rate (%)
C. maculates	A	18	56.2
	B	29	49.8
	C	44	49.4
	Control	88	100
LSD value		9.577 *	7.194 *
* (P<0.05).			

A. Male treated X female treated.
B. Male non-treated X female treated.
C. Males treated X non- treated females.

CONCLUSION

The present study revealed the efficacy of algae extract of *Cladophora glumerata* against some of the biological aspects of *Callosobruchus maculates* . due to the biologically active compounds (table 1). We observe from the results of the experiments the obvious effect of the extract on the percentage mortality of egg in table 2, and also the decrease in adult fertility in egg production after treatment with extract as compared to the control treatment Table 3 This result agrees with [20] , have found the fertility and hatchability of *Dysdercus cingulatus* reduced by used the methanolic extracts algae of *Padina pavonica* and *Sargassum wightii*. The effect of these extracts due to the compounds of alkaloids and other active compound table (1) that act as feeders leading to the destruction of insects, or may be due to the effect of the extracts to their deadly impact on the methods of contact with the surface of the body of the insect and to enter by the respiratory openings affect the nervous system and digestive system in addition to their mortality effect, have an effect on hormones that decline egg rate [21].Saponins play an important role in increased mortality and decreased reproduction in pest insects. The mode of action of saponins in insects demonstrates a block of the uptake of sterols, the insects cannot synthesize sterol structures by themselves [22] and other possibility is that saponins are toxic to insect because of their membrane per mobilizing. is increase the permeability of plasma membranes, and they are known to reason dissociation [23].

We conclude from the present study that it is possible to use the algae extracts in the integrated control programs of this insect to be an easy way of managing the pest and protecting the seeds of the beans or reduce the damage that caused by the infection.

REFERENCES:

1. Cardon,C.; Fam,Z.;Saddek,I.;Bhara and Bushara, A. (2003).Field guide to major insect pests of faba in the Nile Valley.Information Bulletin No.2International center for Agriculture in the Dry.Areas(ICARDA) 38-47.
2. Baidoo, P. K., Mochiah, M. B. and Owusuakyaw, M. (2010). The effect of time of harvest on the damage caused by the cowpea weevil Callosobruchus maculatus (Fab.) (Coleoptera: Bruchidae). Journal of Stored Products and Postharvest Research, 1(3): 24 – 28.
3. Dugje, I. Y., OmoiguiI, L. O. Ekeleme, F., Kamara, A. Y. and Ajeigbe, H. (2009). Farmers' Guide to Cowpea Production in West Africa. IITA, Ibadan, Nigeria.
4. Elzen, G.W., Hardee, D.D., 2003. United States Department of Agricultural-Agricultural Research Service research on managing insect resistance to insecticides. Pest Manag. Sci. 59, 770-776.
5. Kolanjinathan,K.; Ganesh, P.and Saranraj,P.(2014). Pharmacological Importance of Seaweeds: A Review. World Journal of Fish and Marine Sciences 6 (1): 01-15.
6. Gnanamanickam, S.S. (2002). Biological Control of Crop Diseases. New York. Basel: Marcel Dekker, Inc., 15 pp.
7. Van Den Hoek, C. (1982): A taxonom ic revision of the American species of *Cladophora* (Chlorophyceae) in the North Atlantic Ocean and their geographic distribution. - Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Tweede Sect. 78: 236 pp.
8. Rothe , Julie . ; Hays, Dirk and Benemann, John. (2012). Macro-algae (Seaweeds) .TechnologyandCultures,30, 561-583.
9. Auer, M.T.; Tomlinson, L.M.; Higgins, S.N.; Malkin, S.Y.; Howell, E.T. and Bootsma ,H.A. (2010). Great Lakes *Cladophora* in the 21st century: same alga-different ecosystem. Journal of Great Lakes Research. 36(2):248-255.
10. Dodds, W.K. (1991). Factors associated with dominance of the filamentous green alga *Cladophora glomerata*.Wat. Res. 25(11):1125–113.
11. John DM, Whitton BA, Brook AJ. The freshwater algae of the British Isles. An identification guide to freshwater and terrestrial algae. Cambridge University Press, Cambridge 2002.
12. Prescott GW. Algae of the Western Great Lakes area. W.C. Brown Co., Dubuque, Iowa 1962.
13. Harborne, J.B., (1973): Phytochemical methods, London. Chapman and Hall, Ltd., Pp. 49-188.
14. Trease, G.E., Evans, W.C., (1989): Pharmacognsy. 11th Edn.,BrailliarTiridel Can. Macmillan Publishers
15. Mansuya, Periasamy; Aruna ,Pandurangan; Sridhar ,Sekaran; Kumar , Jebamalai Suresh and Babu ,Sarangam. (2010). Antibacterial Activity and Qualitative Phytochemical Analysis of Selected Seaweeds from Gulf of Mannar Region. Journal of Experimental Sciences Vol. 1, Issue 8, Pages 23-26.
16. Güven, K., Percot, A. and Sezik, E. (2010) Alkaloids in marine algae. Marine Drugs, 8, 269-284.
17. Alaa A. A. Al-kemawee and Dina Y. M.Yousif (2017). Detection of Molds which Present in Domestic's Refrigerators in Baghdad City. J. of Global Pharma Technology. 06(9):77-81.
18. Dina Y. M.Yousif and Ahmed S. Dwaish (2016). Activity of two green algae (*zygnemastellinum* and *hydrodictyon reticulatum*) extracts against some phytopathogenic fungi. ejbps, Volume 3, Issue 10, 45-48.

19. Dwaish ,Ahmed. S., Yousif, Y. M. Dina. And Lefta,. N. Siham (2016). "use of *spirogyra* sp. Extract against multidrug resistant bacterial pathogens." International Journal of Advanced Research , Volume 4, Issue 7, 575-579.
20. Asharaja, K. Sahayaraj, 2013A. Screening of insecticidal activity of brown macroalgal extracts against *Dysdercus cingulatus* (Fab.) (Hemiptera:Pyrrhocoridae) J. Biopest., 6 (2013), pp. 193-203
21. Halawa , Z.A. ,Mohamed , R.A. and El-Kashlan, I. I. (1998). Labaratory evaluation of some plant and insecticides against beetle Callosobruchus maculatus infesting stoored product . Egypt. J.Agr. Res. 79(1): 85-93.
22. Belled X., Martin D. & Piulachs M.D. (2005). The mevalonate pathway and the synthesis of juvenile hormone in insects. Annual Review of Entomology 50:181-199.
23. Francis G., Kerem Z., Makkar H. & Becker K. (2002). The biological action of saponins in animal systems: a review. Journal of Nutrition 88:587-605.
24. Harbone, J.B.(1984). Phytochemical methods .Chapman and Hall. New York2nd ed . 288p
25. Tellez, M.R.; Schrader, K.K. and Kobaisy, M. (2001).Volatile components of the cyanobacterium *Oscillatoria perornata (Skuja*). J. Agric. Food Chem., 49: 5989-5992.
26. Yuvaraj, N.;Kanmani,P. ; Satishkumar, R.;Paari, K.; Pattukumar ,V. and Arul,V.(2011).Extraction, Purification and Partial Characterization of *Cladophora glomerata* Against Multidrug Resistant Human Pathogen *Acinetobacter baumannii* and Fish Pathogens.World Journal of Fish and Marine Sciences 3 (1): 51-57, 20