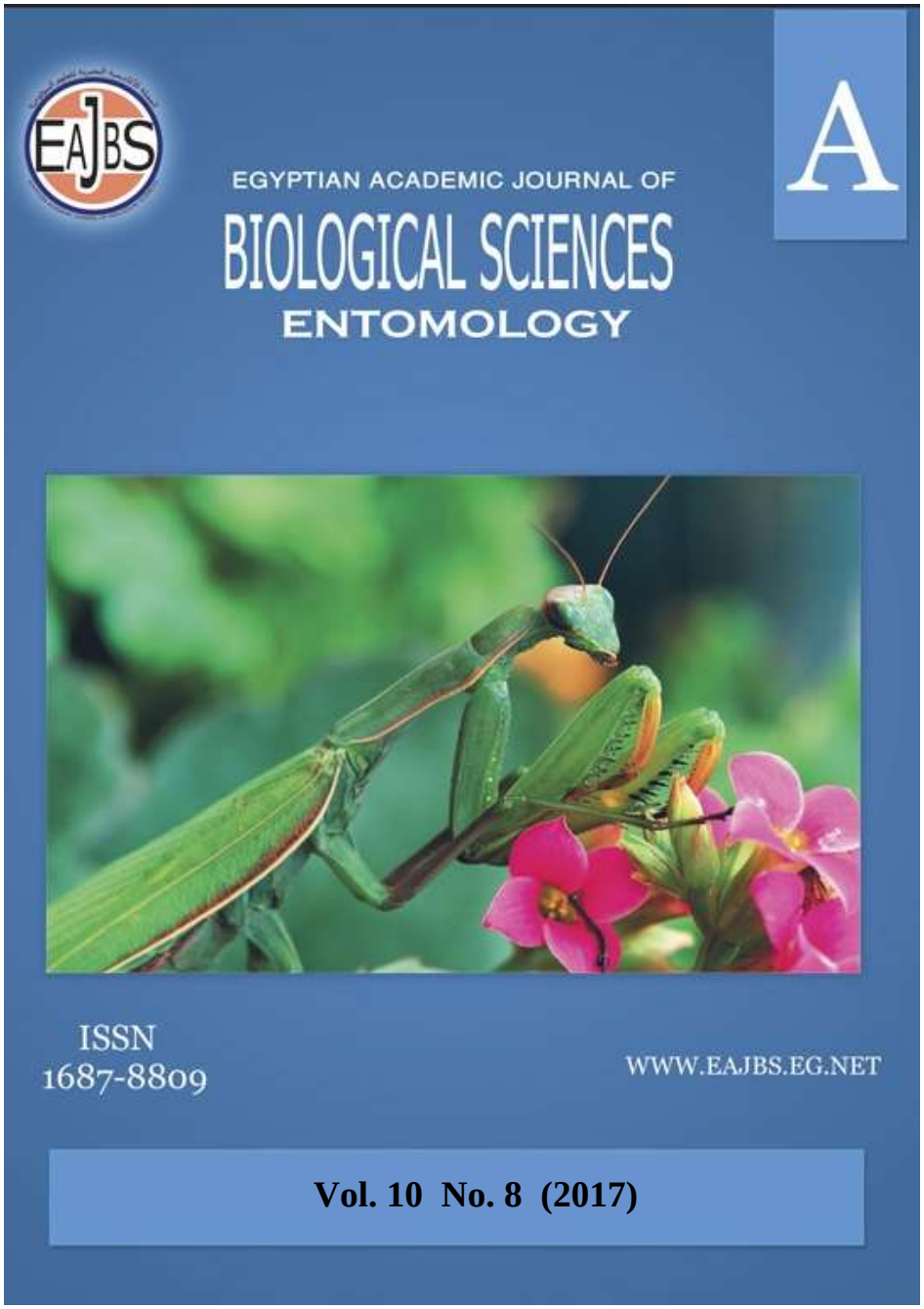

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Evaluation of novel natural product insecticide from The Egyptian Red Sea coast

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ABSTRACT

The present work aims to collect the most dominant marine soft corals: *Sarcophyton glaucum* from the Egyptian Red Sea coast, Gulf of Suez, Al- Ain Elsokhna. Evaluating their potential as insecticides and time of exposure against *Sitophilus oryzae* adults (Coleoptera: curculionidae). Three different solvents (ethanol, chloroform and aqueous extracts of soft corals *Sarcophyton glaucum* and five concentrations as (20, 200, 500, 1000 and 2000 ppm.) were used in all experiments. Screening analysis of active materials of soft corals *Sarcophyton glaucum* extracted by ethanol, chloroform and distilled water were terpene, alkaloids and tannins, respectively. The results of this work indicated that the ethanol extracts of *Sarcophyton glaucum* were achieved the highest mortality rate 100% against adults of *S. oryzae* at high concentration 2000 ppm. LC50 and LC95 values were recorded as 63.18 and 967.1 ppm. respectively, followed by concentration of ethanol extract at 1000 ppm after 96h. Meanwhile, chloroform achieved high mortality rate at 83.33% . Moreover, LC50 and LC95 were recorded at 166.9 and 3200.0 ppm., respectively. On other hands, mortality percentage by using aqueous extract of *S.glaucum* was recorded the lowest mortality rate of 63.33%. Moreover, LC50 and LC95 values were recorded 1036.0 and at 5550.0 ppm respectively, . Due to these results, we concluded that effects ethanol extracts from the soft corals *Sarcophyton glaucum* on *S. oryzae* had potential insecticidal activity followed by chloroform extract and at last came aqueous extract. Moreover, The mortality percentages were increased by increasing the concentration and time of exposure. These extracts of soft coral *S. glaucum* may be promising as a novel natural product which could be used as pesticides against *S. oryzae* adults. So, I would like to recommend these novel products from soft corals to be used, because they are considered eco-friendly insecticides on insects and do not affect on humans, animals and plants and prevent the environmental pollutions

INTRODUCTION

The marine environment is the greatest biome of bioactive natural products, which produce several novel structures with special biological properties. These products may not be found from terrestrial origins. The Red Sea is one of the most marine bio-diverse ecosystems on the earth with high numbers of endemic of marine organisms. The Red sea has many types of soft corals which may reach up to 180

species. Many Known soft corals species worldwide, approximately 40% are endemic to the Red Sea (Edwards1987). Soft corals of the genus Sarcophyton (family Alcyoniidae) are particularly rich in cembranoids diterpenes, Triterpenoids, Tetratepenoids, Ceramide and Cerebrosides. Cembranoids contain 14-membered macro cyclic skeletons and exhibit a wide range of biological activities including anti-tumor, neuro-protective, antimicrobial, calcium-antagonistic, and anti-inflammatory activity Hussien & Mohamed (2015). New and many specific target compounds appeared from marine bioactive products to have a potential role as insect pests management (IPM), (Thakur and Muller, 2004). Soft corals are marine invertebrates having a wide range of terpenoid metabolites. These terpenes, mostly cembranoids, represent the main chemical defense for coral against natural predators (Roethle and Trauner 2008**).** Soft corals of the genus *Sarcophyton* (family Alcyoniidae) are rich in cembrane terpenes, (Blunt *et al* 2008) .Cembranoids contain a 14-membered macro cyclic skeleton and show a wide range of biological activities including anti-tumor, neuro-protective, antimicrobial, calcium-antagonistic, and anti-inflammatory activity(Sawant *et al*,. 2006, Wahlberg and Eklund 1992).

Sarcophyton glaucum, known as rough leather coral, is a common leather coral found in the Red. It grows upon seaward slopes, reef flats and in lagoons (Lieske *et al*., 2004). Lin and Yue (2013) reported that eleven sequiterpenes with six skeletons from 1995 to July, 2011, (one is a novel skeleton) were isolated from six species of this genus, i.e. , *Sarcophyton buitendijki*, *S. elegans*, *S. acutangulum*, *S. glaucum, S. ehrenbergi,* and *S. trocheliophorum*. Moreover, in recent years several kinds of research had paid attention toward marine natural products and many studies have recommended that some of the bioactive compounds isolated from marine organisms exhibited insecticidal properties and its metabolic products may be considered alternative sources for vector and agricultural pest control agents. These bioactive metabolites may potentially replacing the existing and highly toxic synthetic insecticides and will play an important role in the future insecticide development program, (Venkateswara *et al.,* 2003).While previous studies have also suggested that some secondary metabolite isolated from marine organisms had been shown to exhibit larvicidal, insecticidal, and IGR (insect growth regulator) activities (Handayani *et.al.* 1998, Ogamino and Nishiyama 2005). Soft coral of the genus *Nephthea* (Alcyonacea, Nephtheidae) has yielded a variety of bioactive sesquiterpenes and diterpenes which exhibited insecticidal activities on polyphagous pest *spodoptera littoralis* **(**Handayani *et al*.,1997). Our study is shed light on the insecticidal effects of ethanol, chloroform and aqueous extracts of soft coral *Sarcophyton glaucum* against the storage pest *Sitophilus oryzae*.

MATERIALS AND METHODS

Insect Culture:

Adults of *S .oryzae* were collected from naturally infested Freeka (green durum wheat *Triticum aestivum*). Insects were reared in the laboratory on clean, un-infested and sterilized Freeka grains. Twenty adults of insects were released in 20 g of Freeka grains which kept in glass jars (21x10 cm) capped with a cotton cloth to ensure ventilation. All the jars were maintained at room temperature 27±10°C and 65 % RH and continuous darkness. All individuals used in the test were 5-7 days old.

Animal Material:

Soft coral samples *Sarcophyton glacum* were collected from the Egyptian Red Sea Gulf of Suez, Al-ain Elsokhna, August, 2015 at a depth of 4-6 m. The soft coral

was identified by Dr. Abdel Hamid Abdel-Rahman Ali Assistant professor at the National Institute of Oceanography and Fishers Suez branch.

Samples of *Sarcophyton glaucum* were taken to Department of Entomology, Faculty of Science, Benha University. Freshly collected sample of *S. glaucum* was immediately frozen and kept at −20 °C until ready for solvent extraction and analysis.

Extraction and Separation:

The Marine *Sarcophyton glaucum* was cut into small pieces, blended and extracted sequentially with Ethanol, chloroform, and distilled water in Botany Department, Faculty of Science ,Benha University. Initially, the *S.glaucum* (100 g) was soaked in 200 ml . Ethanol for 72 h. and filtered through filter paper. The residue was dried and then extracted sequentially with ethanol, chloroform and distilled water solvents, after 72 h. of soaking in each solvent separately. The extracts were condensed separately under reduced pressure by using vacuum evaporator, and the solvent-free crude extracts were collected in glass vials and stored in 4 °C until use. Stock solutions of the three solvent extracts were prepared and then subjected to bioassay screening. The chemical analysis was performed for screening the active materials by using stander of the different solvent procedures (Trease and Evans, 1989 sofowra 1993) Table (1) .

Insects Bioassay:

Five concentrations were prepared 20, 200, 500, 1000 and 2000 ppm of ethanolic, chloroform and aqueous extracts of *S. glaucum* added to 20g. of Freekeh (green durum wheat, *Triticum aestivum*) which kept in glass jars (21x10 cm). The grains in the control contained solvent only no extract .The jars with their contents were gently shaken to ensure thorough admixture of the freekah and treatment extracts. Twenty adult *S. oryzae* were introduced to each of the jars and covered with cotton cloth and tight with rubber bands. Three replicates of the treatments and untreated controls were prepared. The adult mortality was assessed daily by counting the dead adults. Adults were considered dead when probed with sharp objects and there were no responses

Statistical Analysis:

The mortality percentage of *S. oryzae* in each concentration and control was recorded after 24 h of exposure and the mean ± SE for each triplicate were calculated. The data was then subjected to one way ANOVA test using SPSS program version 21.

LC_{50} and LC_{95} were calculated by Probit analysis (Finny, 1971).

RESULTS AND DISCUSSION

This work is focusing on marine natural products as safe pest control method which may help in minimizing the applying of toxic pesticides and their dangerous effects on non-target insect species, livestock, humans and on the environment (Fatope *et al.*, 1993). Previous studies concluded that the marine natural products may act as toxicant and insects growth regulator. These extracts of marine natural products could be marketable due to their low cost, biodegradability, and safety to the environment as pest control agents. Natural product especially, marine products can support chemical control methods or can play role in integrated pest control programs (IPCP). The current study is conducted to evaluate the efficacy of different concentrations of soft coral *S. glaucum* extracts with the time of exposure of insecticidal activity on *Sitophilus oryzae*. Screening analysis results showed that the active materials of soft corals *S. glaucum* were extracted by ethanol, chloroform

and distilled water are terpenes, alkaloids and tannins, respectively, table (1).This result was in agreement with the findings of Kita *et al.*,(2010) & Fukatsu *et al.*,*(*2007) by applying soft coral *S. glaucum*, which have Secondary metabolites are often derived from symbiotic algae and/or symbiotic bacteria.

Table.1: Screening analysis of active materials of *Sarcophyton glaucum* by different solvents extracts (Ethanol, chloroform and distilled water).

Extracted solvent	Active ingredient			
	Terpene	Alkaloides	Taninns	Flavonoides
Ethanol	*****	--	--	--
coloroform	--	*****	--	--
Distilled water	--	--	*****	--

* denote to the presence of active ingredient
-- denote to not the presence of active ingredient

This study of acute toxicity by using different concentrations (20, 200, 500,1000 and 2000 ppm) of ethanolic extract from soft coral *S.glaucum* on adults *Sitophilus oryzae*, after 96h (Fig.1) shows that 100% mortality percentage was achieved at highest concentrations of ethanolic extracts from *S.glaucum* 2000 ppm. And 1000 ppm. Meanwhile, the mortality percentages of other concentrations 500, 200 and 20 ppm. were 80.0 ,76.66 and 43.0% respectively, after 96 h of treatments with LC_{50} at value 63.18ppm. and LC_{95} at value 967.1 ppm While the mortality percentage after 72h. of treatments at concentrations 20, 200, 500,1000 and 2000 ppm. were at values 26.66, 43.33, 56.66,76.66 and 83.33 % respectively ,with LC_{50} at Value 423.5 ppm. and LC_{95} at value 2193.0 ppm Meanwhile, the mortality percentage after 48 h of treatment at the same concentrations was (20, 200, 500,1000 and 2000 ppm.) were at values 16.66, 30.00, 36.66, 50.00 and 53.33%. respectively, with LC_{50} at Value 1502.0 ppm. and LC_{95} at value 5611.0 ppm In addition, the mortality percentage after 24h. of exposure at concentration was (20, 200, 500,1000 and 2000 ppm) were 10.00,16.66, 20.00, 36.66 and40.00. respectively, with LC_{50} at Value 2273.0 and LC_{95} at value 6200 ppm. The mortality percentage between groups were significant $p \leq 0.05$ in relation to control. This toxicity may be revealed to the extract contain active ingredient terpenes. These results are in a good agreement with the previous report by (Gross *et al*.,2004, Sawant *et al.*, 2006)**,** they stated that some of the typical terpenoids are known as chemical defense tools to protect soft corals from its natural enemies, as feeding deterrents or act as a toxicant. Moreover, most of the secondary metabolites extracted from soft corals have been reported to exhibit a wide spectrum of biological activities such as anti-microbial, anti-fungal, anti-viral, anti-fouling (Wei *et al*., 2013). These secondary metabolites have ecological importance to soft corals (Ishii *et al.*, 2010a). In addition (Blunt *et al.*, 2008, Hussien & Mohamed, 2015), reported that soft corals of the genus *Sarcophyton (*family Alcyoniidae) are rich in cembranoids, diterpenes Triterpenoids, Tetratepenoids, Ceramide and Cerebrosides. Cembranoids contain a 14-membered macro cyclic skeleton and exhibit a vast range of biological activities including neuro-protective, antimicrobial and calcium-antagonistic. In the current study, we recorded the mortality percentage increased with increasing the concentration and exposure time. So, the mortality of insects increased with increasing concentration level and time of exposure from 24 h to 96 h (Fig.2)

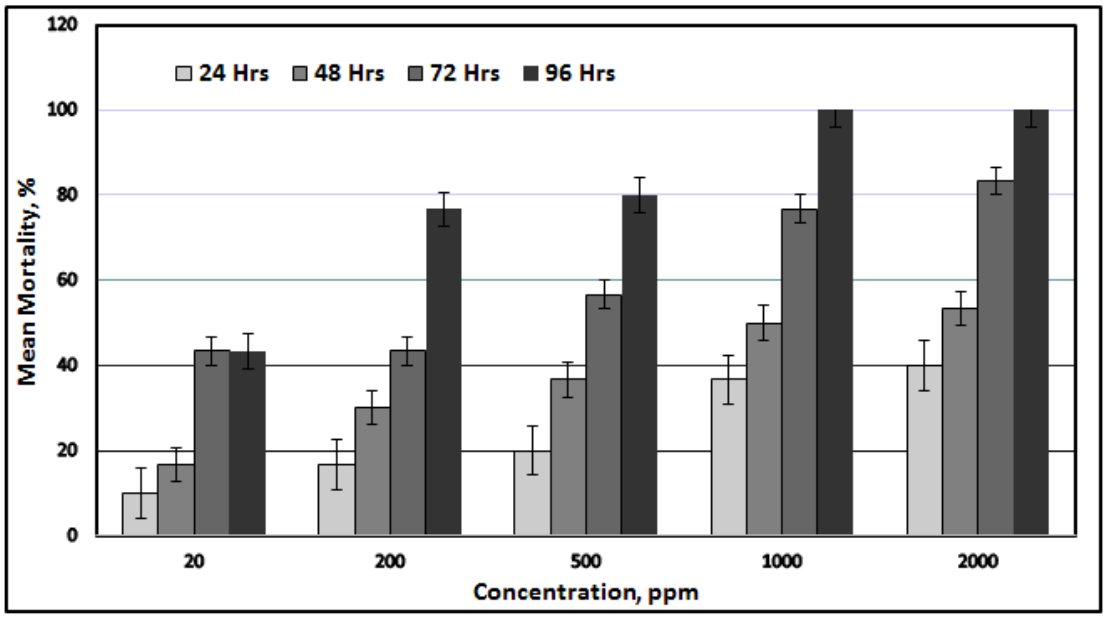

Fig.1. Mean Mortality percentages at different concentrations of ethanolic extract of *Sarcophyton glacum* on the adults of *Sitophilus oryzae*

The Data in (Fig. 2) cleared that the high mortality percentage was achieved at 83.33% and insecticidal activity of chloroform extracts of *S. glaucum* on *S.oryzae* adults may be due to its active ingredient at concentration 2000 ppm followed by the values 70.00, 63.33, 56.66 and 36.66% respectively, at concentrations1000 ,500, 200 and 20 ppm with LC_{50} and LC_{95} values 166.9 ppm and 3200 ppm after 96 h While the mortality percentage after 72 h of treatments were 20.00 , 46.66, 56.66 , 63.66 and 70.00%, at concentrations 20, 200, 500,1000 and 2000 ppm. respectively, with LC_{50} and LC_{95} values 659.7 ppm and 4078.0 ppm respectively. The mortality percentage after 48h. of treatment at the same concentrations were 13.33, 23.33 ,33.33,40.00 and 43.33% respectively, with LC_{50} and LC_{95} values 2119.0 and 6887.0 ppm respectively The mortality percentage after 24 h of exposure were 6.66 ,13.33, 23.33 and 26.66 % respectively, with LC_{50} and LC_{95} at Value 2771.0 and 6985.0 ppm respectively (Table.1).

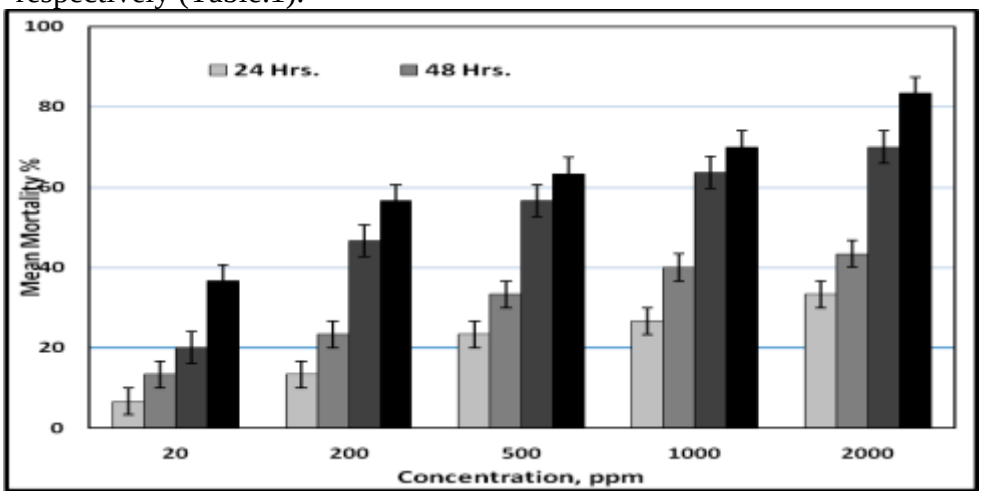

Fig.2. Mean Mortality percentages at different concentration levels of chloroform extract from *Sarcophyton glacum* on the adults of *Sitophilus oryzae*

The Data in cleared that the insecticidal activity of aqueous extracts of *S. glaucum* on *S.oryzae* adults .The mortality percentage (above medium) it was 63.33% at concentration 2000 ppm. followed by 26.66%, at the lowest concentration 20 ppm. The other concentrations 1000 , 500 and200 ppm. were at values 50.00, 46.66 and 40.00% respectively, with LC_{50} and LC_{95} at values 1036.0 and 5550.0 ppm.respectively,. On other hands the mortality percentage after 72h. of treatments were 16.66, 23.33, 36.66 and 43.33%, at concentrations 20, 200, 500,1000 and 2000 ppm. respectively, with LC_{50} and LC_{95} at Values 2224.0 and 6982.0ppm . respectively (Table.2). The mortality percentages after 48h. of treatment at the same concentrations were10.00, 16.66, 23.33,26.66 and 36.66%respectively, with LC_{50} and LC_{95} values 27776.0 and 7610.0ppm.respectively,. The mortality percentage % after 24h. of exposure were 6.66, 10.00 ,13.33 ,16.66 and 23.33 % ,respectively with LC_{50} and LC_{95} at Values 3851.0 and 8708 ppm.

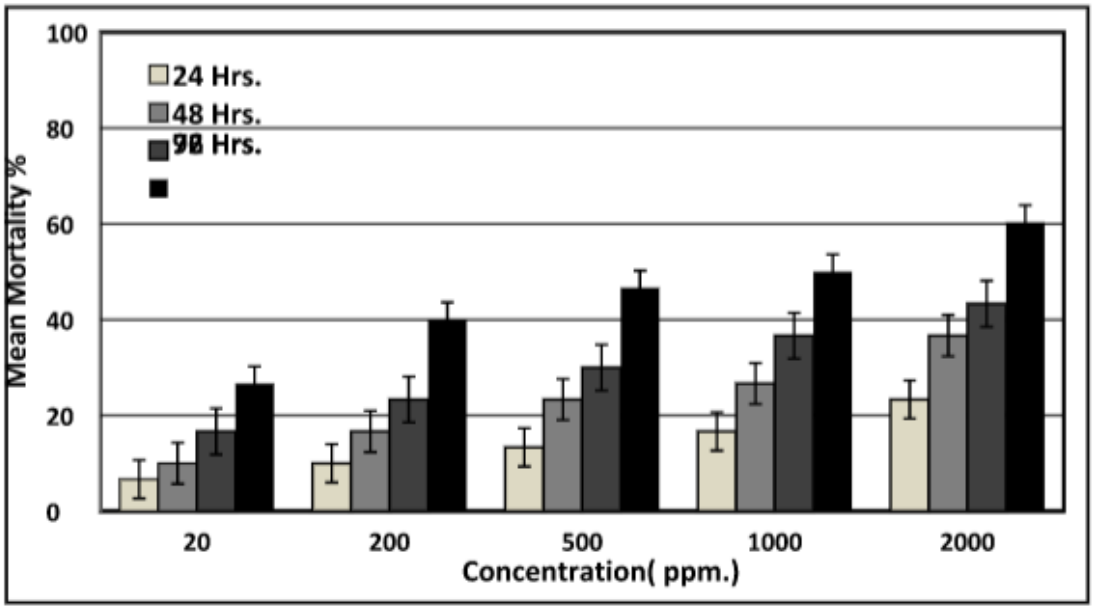

Fig.3 Means Mortality percentages of different concentrations levels of aqueous extract of *Sarcophyton glacum* on the adults of *Sitophilus oryzae*

Table .2 Difference between LC50 and LC95 in *Sarcophyton glaucum* extracts on the adults of *Sitophilus oryza*

Time / hrs.	**Ethanol extract (ppm)**		**Chloroform extract (ppm)**		**Aqueous extracts (ppm)**	
	LC50	LC95	LC50	LC95	LC50	LC95
24 hrs.	2273.0	6200.0	2771.0	6985.0	3851.0	8708.0
48 hrs.	1502.0	5611.0	2119.0	2776.0	2776.0	7610.0
72 hrs.	423.5	2193.0	659.7	2224.0	2224.0	6982.0
96 hrs.	63.18	967.1	166.9	1036.0	1036.0	555.0

The results were recorded the mortality percentages by using the three extracts ethanol, chloroform and aqueous from soft corals *Sarcophyton glaucum*, on *Sitophilus oryzae* adults. We concluded that the ethanolic extract was more effective and achieved the highest mortality rate on *S.oryzae* adults 100%. On the other hand, the aqueous extract was less effective and give the lowest mortality rate (above medium) on *S.oryzae* adults 60.33%. Meanwhile, chloroform achieved high mortality rate less than Ethanol 83.33% at the concentration (2000 ppm.) after 96h. Meanwhile, the mortality percentages at the lowest concentration (20 ppm.) of

ethanol, chloroform and aqueous extracts after 96h. were recorded at 40.00, 33.33and 23.33%. respectively,. These results cleared that the ethanol extracts had potential insecticidal activity followed by chloroform, at last, came aqueous extracts. The *Sitophilus oryzae* is susceptible to the active materials of *Sarcophyton glaucum* extracts and more susceptible to ethanol followed by chloroform and distilled water.

The rate of mortality percentage increased by increasing the concentration and time. In all treatments, the mortality percentages between groups were significant $p \leq 0.05$ in relation to control. This results may be revealed to the presence of terpene , alkaloids and tannins and other chemical contents. Moreover, Sesquiterpenes are secondary metabolites present in many marine organisms including soft coral *e.g*., *Dendronephthya* sp., *Sinularia gardineri, Litophyton arboreum, Sarcophyton trocheliophorum, S. glaucum* and *Parerythropodium m fulvum* (Elkhayat *et al.,* 2014, Ellithey *et al.,* 2013; Al-Footy *et al.,* 2015, Al-Lihaibi *et al.,* 2014).

In addition some authors confirmed the presence of chemicals were isolated from soft corals *Sarcophyton glaucum*. Ne'eman *et al.,* (1974) reported that new diterpene was isolated from soft corals *Sarcophyton glaucum* . This compound was given the name *sarcophine*. It was toxic to mice, rats and guinea pigs and showed strong anti-acetylcholine action on the isolated guinea pig. Sarcophine was also a competitive inhibitor of cholinesterase *in vitro*. Also, Feller *et al.,(*2004), explored, that the chemical content of *S .glaucum* collected from many seas, was thoroughly resulting in the discovery of a large number of cembranoid ,biscembranoid ,sterols, and other secondary metabolites.

Abdel-Lateff *et al.,* (2015) investigated the *S.glaucum* collected from the Red Sea chemically and they found that eleven isoprenoidal metabolites,a new natural cembranoid, sarcophinediol, and seven known cembranoids. Soft corals, sponge and algae investigated by some researcher for bio-insecticides, antibacteria, , antifeedant and other bioactive compounds. Results showed extracts of sponges *Hymanicidon* sp., *Placortis nigra, Theonella* sp., and *Ianthella* sp., soft corals of *Sinularia gravis* and *Sarcophyton cinereu* had antibacterial activity, while those from *Petrosia nigricans, Placs pongia melobesioides, Rhabdastrella of globostellata, Stylissa* of *Carteri, Theonella* sp., were active against *Aedes aegypti* larvae.(Ekowati 2008).

Conclusions:

The present study is the first experimental evidence insecticidal activities of soft coral *Sarcophyton glaucum* marine extracts recorded against adults of *Sitophilus oryzae***.** The mortality percentages by using these active extracts of the novel product of soft coral are promising in *S.oryzae* control. Moreover, are achieved high mortality percentage on *S. oryzae* adults control in comparison with other terrestrial pesticides. The using of these the marine natural product instead of synthetic insecticides could reduce the environmental pollutions.

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ARABIC SUMMERY

تقييم مبيد حشري جديد منتج طبيعيا من ساحل البحر الأحمر المصري

عايدة سعيد كامل

قسم علم الحشرات –كلية العلوم- جامعة بنها – مصر

يهدف هذا العمل لتجميع أشهر الشعاب المرجانية الرخوة (ساركوفيتون جلايكم) من ساحل البحر الأحمر المصري بخليج السويس، لتقييم فاعليته كمبيد حشري والمدة والتركيز المناسبين للقضاء علي سوسة الأرز (سيتوفيلس أوريزي). قمنا بأستخدام ثلاث مستخلصات بأستخدام ثلاث مذيبات لأستخراج المادة الفعاله التي يختبر فاعليتها وهي (الكحول الأيثيلي –الكولوروفورم-الماء المقطر) وكانت المادة الفعاله التي أستخدمت علي التوالي هي (التربين-الألكالويدز-التانيين).ثم قمنا بعمل خمس تركيزات وهي (٢٠٠٠،١٠٠٠،٥٠٠،٢٠٠،٢٠ جزء في المليون) وقد أوضحت نتائج هذا العمل أن مستخلصات الإيثانول من (ساركوفيتون جلايكم) قد حققت أعلى معدل للوفيات ١٠٠٪ ضد البالغين في (سيتوفيلس أوريزي) بتركيز عالٍ ٢٠٠٠ جزء في المليون.تم تسجيل قيم LC_{50} و LC_{95} كما يلي: ٦٣,١٨ و ٩٦٧,١ جزء في المليون على التوالي ، يليه تركيز مستخلص الإيثانول عند ١٠٠٠ جزء من المليون بعد ٩٦ ساعة ، يليه في المرتبه الكلوروفورم وحقق نسبة أماته عاليه حيث كان معدل الوفيات ٨٣,٣٣٪ ، علاوة على ذلك ، تم تسجيل LC_{50} و 166.9وكانت LC_{95} و ٣٢٠٠,٠ جزء في المليون على التوالي. تم استخدام مستخلص مائي من (ساركوفيتون جلايكم) وسجل أدنى معدل وفيات ٦٣,٣٣ ٪ ، وعلاوة على ذلك ، تم تسجيل قيم LC_{50} و 1036.0 LC_{95} وعند ٥٥٥٠,٠ جزء في المليون على التوالي ، . نتيجة لهذه النتائج ، خلصنا إلى أن مستخلصات الإيثانول من الشعاب المرجانية الناعمة (ساركوفيتون جلايكم) على (سيتوفيلس أوريزي) كان لهاأعلي نشاط مبيد حشري يليه الكلوروفورم كان له تأثير عالي ، وفي النهاية جاءت المستخلصات المائية. أعلاوة على ذلك ، زاد معدل الوفيات٪ بزيادة التركيز والوقت. هذه المستخلصات من الشعاب المرجانية الرخوة (ساركوفيتون جلايكم) تبشر بالخير حيث يمكن استخدامها كمنتج طبيعي جديد كمبيدات ضد البالغين (سيتوفيلس أوريزي). لذا ، أود أن أوصي بهذا المنتج الجديد من الشعاب المرجانية الرخوة لأنه يعتبرمن المبيدات الحشرية صديقة للبيئة مستهدفة على الحشرات ولا تؤثر على الإنسان والحيوان والنباتات ومنع التلوث البيئي.