

Toxicological and some chemical effects of *Lurencia papilosa* and *Digenia simplex* algae on *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.) adults

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Abstract: Infestation by stored grains insects reduces the quantity and quality of the stored grain by feeding and reducing its germination ability. Large biomass of seaweeds, enriched with bio-active compounds, is wasted every year along the coasts. Different concentrations (0.0-0.2-0.4-0.6-0.8-1.0 g) of the raw material powder for *Lurencia papilosa* and *Digenia simplex* algae were mixed per 10 g of wheat then infested with *Sitophilus oryzae* and *Rhyzopertha dominica* adult insects. Mortality and progeny reduction percentages of two insects were recorded as well as some biochemical analysis were carried out. Results revealed that *L. papilosa* alga was more efficient in insect toxicity than *D. simplex* alga. *L. papilosa* alga has positive effect on biochemical profiles of *S. oryzae* and *R. dominica* insects as insecticide.

Key words: Bioinsecticide • *Sitophilus oryzae* • *Rhyzopertha dominica* • Algae • Enzymes

INTRODUCTION

Losses of grain in storage due to insects are the final components of the struggle to limit insect losses in agricultural production. Losses caused by insects include not only the direct consumption of kernels [1], but also include accumulations of frass, exuviae, webbing and of insect secretions such as benzoquinone which causes in cancer, failure in liver functions and embryotoxicity [2]. Grain that contains a lot of this insect waste may not be fit for human eating. Changes in the storage environment brought on by insects may result in "hotspots" of warmth and moisture that are favourable for the growth of storage fungi that result in additional losses and aflatoxins [3]. Between five and ten percent of stored product losses worldwide are thought to be attributable to insects. In the tropics, heavier losses could exceed 30 percent and it has been estimated that economic losses caused by stored-product pests can range from 1.25 to 2.5 billion dollars annually in the United States.

Rhyzopertha dominica F. (Coleoptera: Bostrochidae) is a major insect pest of the stored grains in the world. Insects in immature stages may develop inside the grain. Larvae or adults feeding on grain kernels may leave behind dust and thin brown shells or a musty odor are often associated with the infestations of this insect [4].

The worst cereal pest is *Sitophilus oryzae* L. (Coleoptera: Curculionidae). Internal feeders, both the juvenile and adult phases are exceedingly challenging to distinguish. The result was fractured grains with hollow interiors. The weight of grains is said to be reduced by roughly 75% when consumed in large quantities [5].

Chemical pest control, a widespread method for preventing post-harvest losses in stored goods, especially cereal grains, causes a number of issues, including environmental contamination, human toxicity and the creation of insect strains with increased resistance and several other damages [6]. The control of these stored grains and products insects must be developed. The chemical pesticides replacement by green pesticides and other methods not harmful for environment and human or quality of grains or germination [7-9]. To control pests such weeds, insects, fungi, rodents and nematodes, synthetic pesticides are frequently utilized. Due to the growing human population, agricultural production must focus on reducing crop and food loss and supplying enough and wholesome food [10]. Using of non-chemical alternative to fumigation and other methods of chemical control of stored grain insects are required. The excessive use of synthetic pesticides caused serious problems to human health, non-target organisms and ecosystem [11].

Laurencia papillosa and *Digenia simplex* were red algae; Algae are photosynthetic creatures that live in a variety of environments, including severe ecosystems and range in size from microalgae to seaweed [12]. Although most algae are nourishing for mosquito larvae, when consumed in high quantities, some species can kill them. Seaweed phytochemicals provide a natural source of substances for the development of novel insecticides and antimicrobials [13]. The development of extraction techniques has led to an increase in the amount of bioactive chemicals from algae [14]. As phytochemicals isolated from seaweeds may operate against mosquitoes as toxicants, growth regulators, repellents and ovipositional deterrents, seaweeds are significant natural alternatives to insecticides [15, 16]. Algae are unquestionably a safe and effective instrument for crop protection and insect control in both agriculture and public health [17]. Algal extracts, for example, are being developed as bioinsecticides as alternatives to synthetic pesticides, which have detrimental effects on the environment and human health. They have a number of active ingredients that may have biopesticidal activity to control pests and promote sustainable agriculture [18]. Algae-derived bio-insecticides have been touted as a safe and affordable alternative to traditional insecticides [19]. It was found that the plant origin which is used as insecticide affect enzymatic profiles [20].

There aren't many references accessible about marine algae's toxicity against stored grain insects. Therefore, the current study is an effort to use red seaweeds as a safer source of bio pesticides in place of synthetic ones and their impact on biochemical contents of insects.

MATERIALS AND METHODS

Toxicological Studies on Insects

Insect Culture Technique: The insects used in this study were the main stored grain insects; lesser grain borer; *Rhyzopertha dominica* and rice weevil; *Sitophilus oryzae* were reared in Stored Product Pests Research Department, Plant Protection Research Institute, Agricultural Research Center, where a standard culture is maintained without exposure to insecticides for several years and reared at 28±2°C and 65±5 R.H. on whole wheat. The wheat grains were sterilized at a temperature of 55°C for 6 h in order to eliminate any hidden infestation before using [21].

Algae Specie: Two species of red algae *Laurencia papillosa* and *Digenia simplex* are used throughout the present work. These algae were collected from the beach

of Red Sea, Sharm El-Shaikh, Sinai, Egypt during October 2018 and May 2019. Freshly collected alga was repeatedly washed with seawater followed by tap-water to remove any extraneous matters, then identified as *Laurencia papillosa* (C. Agardh) Greville and *Digenia simplex* (Wulfen) C. Agardh, by Prof. Dr. Rawheya Salah El- Din, Professor of Botany, Faculty of Science, Al-Azhar University and Dr. Ehab El-Belely, Ph.D. Applied Phycology, Botany and Microbiology Department, Faculty of Science, Al-Azhar University. The collected algae were dried in the shade, ground and stored in a dark-colored container [22].

Bioassay Test: Wheat samples (10 g) were exposed to various concentrations of each algal powder (0.2-0.4- 0.6-0.8-1.0 g). The wheat grains were hand-shaken both horizontally and vertically after being placed in a 30 g glass jar. In order for each wheat grains to include a thin layer of each conc. coating, followed by the introduction of 25 insects (1- 2 week old) adults from two tested insect species in each glass jar and three replicates for each concentration. The muslin cloth was placed over the jars, they were fastened with rubber bands and they were maintained at 28± 2°C and 65±5 R.H. After 3, 5, 7, 10 and 14 days, mortality results were collected. The similar jars containing untreated grains were used as a control and kept under the same conditions. Then the adults separated from each jar and kept for 40 days at the same previous conditions to record the number of emerged adults (F₁) and calculate the reduction of adult emerged %. Two parameters were recorded mortality percentage and adult emergence of F₁, [9].

Reduction in F₁ = (no. of adult emerged in control- no. of adult emerged in treatment/no. of adult emerged in control) X 100.

Biochemical Analysis for Adult Insects: The two insects were treated with *L. papillosa* alga for 7 days by Lc_{50%} concentration. The treated and untreated (control samples) adult insects separated and homogenized for biochemical analysis in a chilled glass Teflon tissue homogenizer (ST-2 Mechanic-Preczyina, Poland). After homogenation, supernatants were kept in a deep freezer at -20°C till use for biochemical assays. Double beam ultraviolet / visible spectrophotometer (spectronic 1201, Milton Roy Co., USA) was used to measure absorbance of colored substances or metabolic compounds.

Total Proteins: Total proteins were determined by the method of Bradford [23].

Total Carbohydrates: Total carbohydrates were estimated in acid extract of sample by the phenol-sulphuric acid reaction of Dubios *et al.*, [24]. Total carbohydrates were extracted and prepared for assay according to Crompton and Birt [25].

Lactate Dehydrogenase Catalyzes: The method described here is derived from the formulation recommended by the German Society for clinical chemistry [26].

Phenol Oxidase: Phenoloxidase activity was determined according to a modification of Ishaaya [27].

Quantitative Determination of Peroxidase: Peroxidase activity was determined according the procedure given by Hammerschmidt *et al.*, [28].

Statistical Analysis: The results of bioassay were statistically analysis using the probit analysis software program Ldp Line model "Ehab soft" Bakr [29]. Data were analysed by one-way ANOVA using Proc ANOVA in SAS [30]. Means for 3 replicates were compared by least significant difference (LSD; P < 0.05) in the same programme (F test for bioassay and T test for biochemical analysis).

RESULTS AND DISCUSSION

Bioassay Test on Adult Insects: Table 1 showed the use of the raw material powder for algae *L. papilosa* to control the rice weevil *S. oryzae* at concentrations of 0.2-0.4-6-0.8-1.0 g per 10 g of wheat, in addition to the control treatment during 14 days. We showed that the higher concentration, the higher death rates for the insect and also, the longer the time, the death rate gradually increased until the death rate reached 100% after 14 days of treatment at a concentration of 1 g per 10 g of wheat. For the concentration of 1 g, the generation emerged was 20 insects and its reduction %

was the highest (72%) compared to the control which was the mortality % was 1.30% after the same period and number of insects in the first generation was 72 insects. Death rates ranged from 72% to 100% after 14 days from mixing the powder with forementioned concentrations. The reduction % of the first generation ranged between 52 % as the lowest value and 72% as the highest value, Fig. 1.

Data in Table 2 showed the use of algae *L. papilosa* to control the lesser grain borer, *R. dominica* with the same concentrations used in the experiment during 14 days. We noted from the results that the higher the concentration and the long duration of mixing, the better results and the higher death rates occurred. The death rate was recorded 100% at the concentration of 1 g per 10 g of wheat per 25 insects, compared to the control, which had a death rate of 1.30% after the same period. As for the generation emerged was 30 insects and its reduction % was (62.96%) for a concentration compared to the control whose number of insects in the generation was 81 insects. Death rates ranged from 60% to 100 % after 14 days from mixing the powder with forementioned concentrations. The reduction % of the first generation ranged between 51.40 % as the lowest value and 62.96 % as the highest value, Fig. 1.

Table 3 showed the use of algae powder of *D. simplex* in the control of the rice weevil, *S. oryzae* using concentrations of 0.2-0.4-0.6-0.8-1 g/ 10 g wheat grains after mixing them for periods of up to 14 days from the insect infestation as well as the number of insects resulting from the first generation and the percentage of its reduction compared to the control treatment. From the results, it becomes clear that the longer the mixing time and the higher concentration of the powder, the higher death rates and reach their highest rates after 14 days, which range between 61.33% and 100%. The reduction % of the first generation ranged between 56 % as the lowest value and 76% as the highest value, Fig. 1.

Table 1: Insecticidal effect of *L. papilosa* alga on *S. oryzae* adults.

g/10 g wheat	Mortality (%) after indicated periods (day)					F	P	Number of F ₁ progeny	Reduction %
	3	5	7	10	14				
0.2	14.60±0.01 ^{Ee}	48±0.04 ^{Dd}	56±0.04 ^{Ec}	62.66±0.01 ^{Eb}	72±0.02 ^{Da}	46.81	<0.0001	±351.7 ^B	52
0.4	21.33±0.01 ^{De}	57±0.01 ^{Cd}	65.33±0.01 ^{Dc}	73.33±0.01 ^{Db}	84±0.023 ^{Ca}	458.6	<0.0001	33±0.6 ^C	56
0.6	26.66±0.01 ^{Cc}	65.30±0.01 ^{Bd}	70.66±0.01 ^{Cc}	81.33±0.01 ^{Cb}	92±0.023 ^{Ba}	541.2	<0.0001	27±0.6 ^D	62
0.8	33.33±0.01 ^{Be}	66.66±0.01 ^{Bd}	74.66±0.01 ^{Bc}	84±0.023 ^{Bb}	93.33±0.01 ^{Ba}	539.5	<0.0001	24±0.7 ^E	67
1	38.66±0.02 ^{Ae}	70.66±0.01 ^{Ad}	77.33±0.01 ^{Ac}	88.40±0.023 ^{Ab}	100±0 ^{Aa}	745	<0.0001	20±1.7 ^F	72
Control	0±0 ^{Fb}	0±0 ^{Eb}	0±0 ^{Fb}	1.30±0.023 ^{Fa}	1.30±0.02 ^{Ea}	6.982	0.0028	72±1.5 ^A	-
F	81.43	147.1	178.5	356.7	414.7				
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001				

Means followed by different letters are significantly different from each other at P<0.05 (Tukey test).

Capital letters represent differences between columns and small letters represent differences between rows.

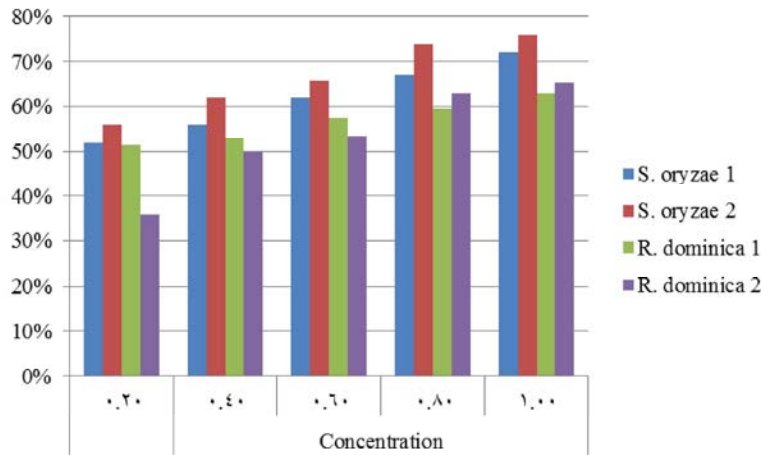


Fig. 1: Reduction % of F₁ progeny of *S. oryzae* and *R. dominica* adults after treated with *L. papilosa* (1) and *D. simplex* (2) algae.

Table 2: Insecticidal effect of *L. papilosa* algae on *R. dominica* adults.

g/10 g wheat	Mortality (%) after indicated periods (day)						F	P	Number of F ₁ progeny	Reduction %
	3	5	7	10	14					
0.2	4±0 ^{Dc}	14.60±0.01 ^{Dd}	21.33±0.01 ^{Dc}	41.33±0.01 ^{Eb}	60±0.02 ^{Ea}	271.4	<0.0001	39±2 ^B	51.40	
0.4	18.66±0.01 ^{De}	25.30±0.02 ^{Cd}	41.33±0.01 ^{Cc}	61.33±0.01 ^{Db}	77.33±0.01 ^{Da}	310.1	<0.0001	38±2.9 ^B	52.84	
0.6	25.33±0.02 ^{Cc}	41.30±0.01 ^{Bd}	50.66±0.01 ^{Bc}	68±0.06 ^{Cb}	88±0.02 ^{Ca}	98.15	<0.0001	34±5.0 ^C	57.50	
0.8	34.66±0.01 ^{Be}	46.66±0.01 ^{ABd}	53.33±0.01 ^{ABc}	86.66±0.03 ^{Bb}	93.33±0.01 ^{Ba}	330	<0.0001	33±1.53 ^D	59.50	
1	37.33±0.01 ^{Ac}	49.33±0.01 ^{Ad}	57.33±0.01 ^{Ac}	94.66±0.01 ^{Ab}	100±0 ^{Aa}	1060	<0.0001	30±1.53 ^E	62.96	
Control	0±0 ^{Eb}	0±0 ^{Eb}	0±0 ^{Eb}	1.30±0.013 ^{Ea}	1.30±0.013 ^{Ea}	6.982	0.0028	81±2.31 ^A	-	
F	115.9	163.3	337.4	5.003	497.8					
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001					

Means followed by different letters are significantly different from each other at P<0.05 (Tukey test). Capital letters represent differences between columns and small letters represent differences between rows.

Table 3: Insecticidal effect of *D. simplex* algae on *S. oryzae* adults.

g/10 g wheat	Mortality (%) after indicated periods (day)					F	P	Number of F ₁ progeny	Reduction %
	3	5	7	10	14				
0.2	17.33±0.01 ^{Ee}	33.33±0.01 ^{Dd}	41.33±0.01 ^{Dc}	46.66±0.01 ^{Eb}	61.33±0.01 ^{Da}	274.8	<0.0001	32±1.15 ^A	56
0.4	21.33±0.01 ^{De}	36±0.02 ^{Cd}	45.33±0.02 ^{Cc}	50.66±0.01 ^{Db}	68±0.02 ^{Ca}	137.8	<0.0001	28±1.15 ^B	62
0.6	26.66±0.01 ^{Cd}	41.33±0.01 ^{Bc}	52±0.02 ^{Bb}	57.33±0.01 ^{Cb}	88±0.04 ^{Ba}	148.5	<0.0001	25±1.73 ^{BC}	66
0.8	30.66±0.01 ^{Be}	44±0.02 ^{ABd}	57.33±0.01 ^{ABc}	62.66±0.01 ^{Bb}	96±0.02 ^{Aba}	352.5	<0.0001	21±0.6 ^C	74
1	33.33±0.01 ^{Ac}	46.66±0.01 ^{Ad}	60±0.02 ^{Ac}	72±0.01 ^{Ab}	100±0 ^{Aa}	255.3	<0.0001	17±0.6 ^D	76
Control	0±0 ^{Fb}	0±0 ^{Fb}	0±0 ^{Fb}	1.30±0.01 ^{Fa}	1.30±0.01 ^{Fa}	6.982	0.0028	72±1.6	-
F	98.6	66.31	136.8	258.5	225.8				
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001				

Means followed by different letters are significantly different from each other at P<0.05 (Tukey test). Capital letters represent differences between columns and small letters represent differences between rows.

Table 4 showed the results of toxicity of algae *D. simplex* on the lesser grain borer, *R. dominica* as well as the reduction % of first generation. The results showed that the lowest percentage for death after day 14 was 66.66 % as the highest percentage was 100% as the highest and the reduction % of the first generation ranged between 35.92% and 65.43%, Fig. 1.

Figure 1 cleared that reduction % in F₁ progeny emerged from insects treated with 0.8 and 1.0 concentrations was higher in insects treated by *D. simplex* algae than *L. papilosa* algae while for insects, the reduction % in F₁ progeny was higher in *S. oryzae* than *R. dominica* insect.

Through Table 5 it is resulted that the powder of *L. papilosa* algae was more efficient in insect toxicity as

Table 4: Insecticidal effect of *D. simplex* alga on *R. dominica* adults.

g/10 g wheat	Mortality (%) after indicated periods (day)							F ₁ progeny	Reduction %
	3	5	7	10	14	F	P		
0.2	12±0.02 ^{Dd}	17.33±0.01 ^{Dd}	25.33±0.01 ^{Dc}	37.33±0.01 ^{Eb}	66.66±0.03 ^{Da}	127.6	<0.0001	52±1.15 ^B	35.92
0.4	14.60±0.01 ^{Cc}	21.33±0.01 ^{Cd}	33.33±0.01 ^{Cc}	41.33±0.01 ^{Db}	80±0.02 ^{Ca}	336	<0.0001	41±4.58 ^C	49.87
0.6	18.66±0.01 ^{Bc}	25.33±0.02 ^{BCd}	40±0.02 ^{Bc}	52±0.02 ^{Cb}	92±0.02 ^{Ba}	226.2	<0.0001	38±1.53 ^D	53.58
0.8	21.33±0.01 ^{ABd}	29.33±0.01 ^{Bd}	48±0.01 ^{Ac}	68±0.01 ^{Bb}	96±0.03 ^{ABa}	336.7	<0.0001	30±2.45 ^E	62.96
1	24±0.016 ^{Ac}	38.66±0.013 ^{Ad}	50.66±0.023 ^{Ac}	77.33±0.016 ^{Ab}	100±0 ^{Aa}	450.2	<0.0001	28±1.224 ^E	65.43%
Control	0±0 ^{Fb}	0±0 ^{Fb}	0±0 ^{Fb}	1.30±0.013 ^{Fa}	1.30±0.013 ^{Fa}	6.982	0.0028	81±2.309 ^A	-
F	27.64	71.63	97.53	242.6	271.7				
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001				

Means followed by different letters are significantly different from each other at P<0.05 (Tukey test).

Capital letters represent differences between columns and small letters represent differences between rows.

Table 5: LC_{50%} and LC_{90%} values and their confidence limits for *S. oryzae* and *R. dominica* adults exposed to two algae at 7, 10 and 14 days.

Alga	Insect	day	50% limits			90% limits			X ²	Slope±SE	H
			Conc. (g/10 g)	Lower	Upper	Conc.	Lower	Upper			
<i>L. papilosa</i>	<i>S. oryzae</i>	7	0.13	0.03	0.23	4.28	1.93	56.71	0.014	0.85±0.23	0.005
		10	0.11	0.04	0.17	1.32	0.92	2.88	0.22	1.19±0.25	0.075
		14	0.09	0.04	0.14	0.55	0.45	0.73	6.45	1.65±0.29	2.15
	<i>R. dominica</i>	7	0.66	0.55	0.84	5.68	3.04	20.00	1.39	1.37±0.24	0.466
		10	0.28	0.11	0.35	1.03	0.89	3.27	9.16	2.26±0.25	3.05
		14	0.15	-	-	0.75	-	-	20.65	1.85±0.29	6.88
<i>D. simplex</i>	<i>S. oryzae</i>	7	0.47	-	-	31.76	-	-	0.50	0.70±0.23	0.168
		10	0.30	0.15	0.42	9.00	3.29	201.5	2.26	0.87±0.23	0.75
		14	0.16	-	-	0.75	-	-	10.55	1.92±0.27	3.52
	<i>R. dominica</i>	7	0.98	0.73	1.82	18.28	5.86	398.53	0.33	1.01±0.24	0.111
		10	0.54	-	-	4.19	-	-	8.88	1.32±0.23	2.96
		14	0.12	-	-	0.62	-	-	14.45	1.84±0.29	4.81

mortality rates than algae *D. simplex*, where the low weight used indicates the efficiency of the material. Also, at LC_{50%} and LC_{90%}, *S. oryzae* was more sensitive of *L. papilosa* alga than *R. dominica* adults while *R. dominica* was more sensitive of *D. simplex* alga than *S. oryzae* after 14 days treated. From the results, found that both algae have a toxic effect on the two insects.

The reason for the toxicity of two species of algae to insects is due to their chemical composition, which may causes the insect to no want to feed or to repellent from food. And perhaps the presence of dehumidifying substances that make the insect lose its moisture and dryness, whether through feeding or by entering the powder particles into the respiratory stomata of the insect, which makes it drier and prevents it from breathing as well as in diatomaceous soil powder, thus stopping the vital and physiological processes in the body. The variation in the effect of components on one insect over the other is attributable to the insect's tolerance level and resistance to the substance. This is in line with the discovery that the toxicity of the tested algal extracts to *Culex pipiens*

larvae may be related to the release of reactive oxygen species, which stimulated superoxide dismutase activity and led to high levels of superoxides and low catalase activity, resulting in an oxidative imbalance and excessive accumulation of reactive molecules [31]. These reactive chemicals oxidise the proteins and lipids that are connected to the membrane, destroying the integrity and functions of the membrane. They also damage the nucleic acids and DNA, impairing cellular activity and killing the insects [32]. Ismail *et al.* [33] recorded that the activity of seaweeds differed due to the extract solvent and seaweed species. Ishii *et al.* [34] according to their study, laurinterol also displayed insecticidal efficacy against the termite *Reticulitermessperatus*, repellent activity against the maize weevil *Sitophilus zeamais* and an inhibitory impact on acetylcholinesterase (AChE). The red algal genus *Laurencia* may be a good source of bioactive natural products with insecticidal activity, according to this first report on laurinterol's insecticidal and repellent properties, which raises the possibility that laurinterol may one day be used to create new repellents and/or insecticides for controlling pests like termites and

Table 6: Biochemical analysis of two adult insects treated for 7 days with LC_{50%} of *L. papilosa* alga

	Parameter	Total protein (mg/g.b.wt)	Total carbohydrate (mg/g.b.wt)	LDH (μ /g.b.wt)	Phenol oxidase (Δ O.D.units /min/g.b.wt)	Peroxidase (Δ O.D./min/g.b.wt)
<i>S. oryzae</i>	Treatment	25.9 \pm 0.49 ^B	19.07 \pm 0.87 ^A	2341 \pm 47.94 ^A	2.54 \pm 0.13 ^B	3.04 \pm 0.09 ^A
	Control	29 \pm 0.55 ^A	18.27 \pm 0.38 ^A	2269 \pm 44.76 ^A	3.78 \pm 0.27 ^A	3.08 \pm 0.15 ^A
<i>R. dominica</i>	Treatment	26.63 \pm 0.84 ^b	14.56 \pm 0.76 ^b	661 \pm 20.12 ^a	6.43 \pm 0.23 ^a	20.5 \pm 0.76 ^b
	Control	32.96 \pm 1.44 ^a	19.36 \pm 0.95 ^a	597 \pm 6.50 ^b	7 \pm 0.23 ^a	27.46 \pm 1.07 ^a

The same letter in the same column means the non-significant between treatment and control and vice versa.

Capital letters represent differences between treatment and control for *S. oryzae* and small letters represent differences between treatment and control for *R. dominica* insects.

stored-product insects. The findings of this study may aid future investigations into the isolation of natural compounds with insecticidal activity from marine species.

(Elbrense and Gheda) [35] resulted that all seaweed extracts exhibited insecticidal and antifeedant activities and third instar larvae of *Spodoptera littoralis* were the most susceptible for extracts application. Ethanolic extract of *Turbinariaturbinata* caused the highest mortality (83.33 \pm 1.92%) in third instar larvae of *S. littoralis* while *Sargassum. Acinarium* caused the highest mortality (53.33 \pm 6.93%) in the fifth instar larvae of *S. littoralis* and (30.00 \pm 3.33%) in adults of *R. dominica*. Whereas, the methanolic extract of *Pterocladia capillacea* was the most feeding inhibitor against the tested insects. There were direct relationships between the mortality percentage and both seaweed concentrations and exposure time. Gas Chromatography-Mass Spectrometry analysis of seaweed extracts showed bio-active compounds mainly Diisooctyl phthalate, terpenoides, decane, phenolics and fatty acids. Therefore, seaweed extracts can be used as eco-friendly biopesticides for control of *S. littoralis* and *R. dominica* and can be recommended to be involved in the pest management programs. Additionally, compared to green and brown algae, red algae are rich in polyphenolic and terpene chemicals. These triterpenes block the protein necessary for the transfer of cholesterol during larval development, which results in larval mortality [36, 37]. In addition to seasonal fluctuations, which have an impact on the chemical makeup of the bioactive metabolites and their production, ecological and geographic factors may also contribute to the bioactivity differences between species within the same division [38-40]. The phenolic compounds in natural materials' extracts have various biological activity [41- 44]. In the study of Yu *et al.* [45] the larvicidal activity of these algal extracts might be due to various bioactive compounds, including phlorotannins, Algae contain halogenated substances, amino acids, alkaloids, polysaccharides, terpenoids, saponins, flavonoids and phenolics. However, the amount of marine algae varies depending on the species, location, weather and season [46]. In addition to causing larval mortality, the

metabolites derived from algae also interfered with the larvae's proper development and metamorphosis into pupae. The presence of poisonous substances like farnesyl acetone and plastoquinones, which block the AChE enzyme, may be the reason why red algal extracts are so potent. Additionally, compared to green and brown algae, red algae are rich in polyphenolic and terpene chemicals. These triterpenes block the protein necessary for the transfer of cholesterol during larval development, which results in larval mortality [36, 37]. It is important to extract and characterise the active ingredients. In general, terpenes, alkaloids and polyphenolic chemicals are said to be concentrated in red seaweeds [47].

Biochemical Analysis of Adult Insects: The effect of *L. papilosa* alga with concentration that caused 50% death of the *S. oryzae* and *R. dominica* adults after 7 days treated were evident in Table 6. From the results noticed that *S. oryzae* insects treated with *L. papilosa* alga had total protein content, phenol oxidase and peroxidase lower than control with significant differences between treated and control for protein and phenol oxidase but without significant differences for peroxidase. The total carbohydrates and LDH were higher in treated insects than control but without significant differences between them. In *R. dominica* insects treated with *L. papilosa* alga, there were decreased in each of total proteins, total carbohydrates, phenol oxidase and peroxidase than the control. The statistical analysis showed that there were significant differences between treated and control insects in all biochemical parameters except in phenol oxidase. LDH increased in treated insect than control with significant differences between them. In general, the same trend observed as a mode of action for *L. papilosa* alga on two insects in biochemical contents except the total carbohydrate was different. The decrease in total proteins may be due to the fact that algae acted as an antifeedant or repellent, so the content of protein, which are the important element in the components of the body, were decreased and since enzymes are considered proteins, they were also decreased accordingly, the result goes with line of Ghoneim

et al. [48] and Qari *et al.* [49] recorded that change in protein content reflect the balance between degradation and synthesis of functional nutrients and response to detoxified for insecticide, or due to the enzyme act as antioxidant and arising from aerobic metabolism and dietary prooxidants which can damage proteins, lipids and other important macromolecules or due to terpenes components in alga block the protein necessary for the transfer of cholesterol during larval development, which results in larval mortality [36, 37]. So the *L. papilosa* alga had the same effect as an insecticide. On the other hand, LDH increased in treated insect than control this go in the same trend of Brown *et al.* [50] who stated that LDH expressed in high concentration when cells exposed to damage.

CONCLUSION

- Red marine algae can be used as natural insecticide.
- The alga of *L. papilosa* was more efficient in insect toxicity than alga *D. simplex*.
- *S. oryzae* adult had sensitivity for *L. papilosa* alga while *R. dominica* insect had a sensitivity for *D. simplex* alga.
- The reduction percentages of the first generation were higher in the *D. simplex* alga powder than the second alga for both insects and were higher in *S. oryzae* than *R. dominica* especially with the use of higher concentrations.
- *L. papilosa* algae had positive mode of action on *S. oryzae* and *R. dominica* insect as insecticide.

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