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Insecticidal Potential of Botanicals from Red Seaweeds against Stored Grain Pests, Rice Weevil (*Sitophilus oryzae* L.) and Cowpea Weevil (*Callosobruchus maculatus* Fab.)

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RB performed toxicology test and prepared manuscript. RMT reared insect pests, set experiment and interpreted results. SAMA interpreted result, applied statistics and reviewed manuscript. MR designed the experiment, provide seaweeds extracts, finalized manuscript.

Key words
Sitophilus oryzae, Callosobruchus maculatus, Laurencia karachiana, Gracilaria foliifera, Asparagopsis taxiformis, Jania rubens

ABSTRACT

Marine botanicals are enriched with natural bioactive compounds have been utilized in the current study to alleviate the stored grain pests infestation. Four species of red seaweeds *Asparagopsis taxiformis, Laurencia karachiana, Gracilaria foliifera,* and *Jania rubens* found abundantly along the Karachi coast, Pakistan were collected in the year 2015-18. Samples were extracted using a Soxhlet extraction method with solvents of varying polarity (hexane, dichloromethane, and methanol). Five different concentrations of the extracts were subjected to the toxic assessment against two species of stored grain pests (*Sitophilus oryzae* and *Callosobruchus maculatus*). Results obtained from experimental trail proved that the *C. maculatus* were susceptible with higher adult mortality than *S. oryzae*. The highest toxic effect was induced by dichloromethane obtained extract from *A. taxiformis* (DA) against *C. maculatus* (LC$_{50}$ 1.15 mg/cm^2) after 24 h exposure and *S. oryzae* although resist for one day showed (LC$_{50}$ 1.44 mg/cm^2) after 48 h. Neurotoxic effects were also determined after 12 and 24 h. *L. karachiana* was the second in toxicity against *C. maculatus* and *S. oryzae* population. In addition, all treatments of *A. taxiformis* and *L. karachiana* significantly reduced the eggs laying by *C. maculatus* counted after 96 h of treatment. More than 70% mortality was also obtained after 96 h exposure at a dose of 2.2 mg/cm^2 with most of the seaweed extracts against *S. oryzae* and *C. maculatus*. While treatment with *J. rubens* and *G. foliifera* provided only low to moderate toxicity to both tested species.

INTRODUCTION

Apart from insufficient food production needed for completing the human population demand, food security is also a fundamental problem in many developing countries including Pakistan. Advanced technologies have not been introduced in various under developing countries. These technologies include grain dryers, seed pelleting, sensors, nano-techniques, genetic manipulation of cowpeas against bruchids, sealed bags such as Purdue Improved Cowpea Storage 'PICS' and hermetic bags and/or modern storehouses that built on scientific design (Adedire and Ajayi, 2003; Mutungi *et al*., 2014; Afzal *et al*., 2019; Mahto *et al*., 2019; Kpoviessi *et al*., 2019). Considerably, more than 1600 insect species have been known to attack agricultural products starting from post-harvest till the consumers' home (Hagstrum and Subramanyam, 2009; Nawrot and Harmatha, 2012). It is estimated that 10-40% post-harvest damages are caused by the stored grain pests every year, globally (Rajashekar *et al*., 2010). In Pakistan annually loss of cereals is about 5.6 metric tonnes worth $1.7 billion (Afzal *et al*., 2019).

Pakistan has an agricultural based economy and contributes on large scale in the production and export of cereal, grain and other cash crops. Rice is the third major crop of Pakistan. Besides, Pakistan ranks fourth in world's biggest exporter of rice and ranks ten among biggest rice producer of the world (Shah *et al*., 2020). In the year 2017-18, 7.4 million tonnes annual production was reported with 3.84 million metric tonnes rice export fetching $1.889 billion (USDA, 2018). Chickpea production also plays important role in the economy of Pakistan with annual production of 675 thousand tonnes (Kouser *et al*., 2017). The significant cultivation of this staple food is considered

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as the poor man's meat which contains a rich source of protein and carbohydrate fulfilling the human nutritional requirement (Ahmed *et al*., 1991). However, by the last couples of year, crop production is affected by the climate change, drought condition, shortage of water and damages caused by the insects (Rehman *et al*., 2015).

Infestation by coleopteran beetle reduces the quantity of the harvested grain by feeding and also seeds' productivity by reducing its germination ability. Their rapidly growing population contaminates grains with eggs, dead bodies, webbing, toxins, and hairs or by casting skins which increases the grain susceptibility for fungus growth, a further source of diseases. Moreover, proteins secreted by insects are involved in producing allergic reactions to human (Arlian, 2002). Ultimately, the grains damaged by the beetles with holes lose its cultural and commercial values, becoming unfit for the human and animal consumption (Tripathi *et al*., 2009).

Sitophilus oryzae L. also known as rice weevil (Coleoptera: Curculionidae) is the most damaging cereal pest. Both the immature and adult stages are the internal feeder and very difficult to recognize. It lefts grains broken and hollow form inside. Extensive feeding on grains is reported to reduce about 75% of the grain's total weight (Singh *et al*., 2009).

Callosobruchus maculatus Fab. (Coleoptera: Bruchidae) trivially called cow-pea weevil is the serious, multivoltine pest, causing serious damages to stored pulses in Pakistan and many other tropical countries. Bruchid beetles feed on the grains producing holes, which are then used to lay eggs. Often whole grain and these holes are found covered and filled with eggs. Heavy infestation resulting in the severe weight loss and powdering of stored commodities. In a period of 3 to 6 months storage, up to 90% damage can be caused by the *C. maculatus* alone (Ofuya, 1986; Boeke *et al*., 2004).

Control strategy is still based on the fumigation and synthetic pesticides, an environmental hazards. Residues of pesticides cause risk to health of living organisms and real danger to human. To combat the increasing resistance, rate the demand of novel and effective bioactive insecticidal compound has been increased (El-Wakeil, 2013). Therefore, some quick action is needed to be taken to control the destruction of food crop, especially by the stored pests. Thus only by controlling crop pests it can be possible to get the healthy nutritional food. A number of botanicals touted as an antifeedant, toxicants, and repellent against stored grain pests have been reported broadly (Jayasekara *et al*., 2005; Abdelgaleil *et al*., 2009, 2016). Marine algae are rich source of novel bioactive and secondary metabolites possess diverse biological activities (Kolanjinathan *et al*., 2014). More than 2400 natural products have been reported from seaweeds and the largest varieties of natural products are found to be present in Rhodophyta (Red algae) (Domettila *et al*., 2013). Bio-potential of seaweeds against dengue mosquito population (Bibi *et al*., 2020), root rotting fungi and nematodes (Sultana *et al*., 2018), antimicrobial (Ambreen *et al*., 2012), antileishmanial (Sabina and Aliya, 2011), antioxidant (Sabina *et al*., 2006) larvicidal (Hira *et al*., 2017) have been reported. However, few citations are available concerning the toxicity to stored grain pests (Fukuzawa and Masamune, 1981; Abou-Elnaga *et al*., 2011; Rizvi and Shameel, 2003). Large biomass of seaweeds, enriched with bio-active compounds, is wasted every year along the Karachi coast (Dawn News, 2020). Therefore, current study is an approach to utilize red seaweeds as an alternative safer source of bio pesticides to replace synthetic pesticides.

MATERIALS AND METHODS

Insecticidal screening of four red seaweeds *A. taxiformis, G. foliifera, L. karachiana, J. rubens* extracts were experimented against two species of stored grain pests *S. oryzae* and *C. maculatus*

Test insects

For pest infestation, rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) and cowpea weevil, *Callosobrocus maculatus* F. (Coleoptera: Bruchidae), were collected from the Toxicology Laboratory of Department of Zoology, University of Karachi. Pests were reared in laboratory under controlled conditions maintained at 29 ± 4 °C, 50 – 70 % RH. Rice weevil, *S. oryzae* was reared in whole rice and *C. maculatus* was reared in whole lentils. First generation of adults (1 - 4 days old) was used for the toxicity assessment.

Collection of seaweeds

Two species of red seaweeds *Laurencia karachiana* sp. nov (molecularly confirmed Bibi *et al*., 2019) [KUH-SW-No. 1310b] and *Asparagopsis taxiformis* [KUH-SW-No. 1206] were handpicked from French Beach (24°50'15"N 66°49'11"E) and others two *Gracilaria foliifera* [KUH-SW-No. 1261] and *Jania rubens* [KUH-SW-No. 1290] were collected freshly from Hawke's Bay (24°50'34"N 66°53'56"E) of the Karachi coast during the year 2015-18 (Fig. 1). Samples were deposited in the Karachi University Herbarium (KUH), for voucher numbers as mentioned in parenthesis (*vide supra*).

Preparation of seaweed materials and extraction

Seaweeds were washed thoroughly with tap water to remove salt, sand, or epiphytes, and air-dried at room temperature. Samples were grounded and sieved (60 mesh size). Extracts were prepared using Soxhlet extraction method. Powdered seaweed (20g (x4)) was refluxed with solvent (500 ml) for 8 h in Soxhlet flask (250 ml) as per method described in AOAC (1995). Solvents of varying polarity i.e., hexane, dichloromethane, and methanol (Fisher Scientific, Leicestershire, UK) were used. Solvent was evaporated using rotary vacuum evaporator (Eyela, Tokyo, Japan). Extracts were refrigerated in amber vials at 4 ºC till toxicity assessment. Details of the extracts' codes are given in the footnotes of tables.

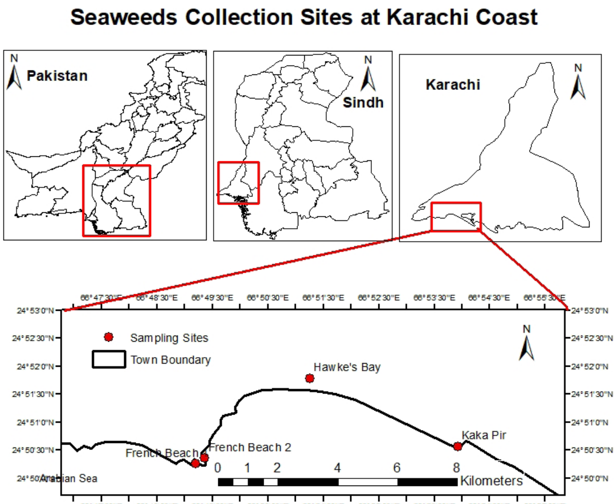

Fig. 1. Study map showing the sampling sites in Karachi coast.

Contact toxicity assay

For toxicity assessment contact toxicity method was applied (Auamcharoen *et al*., 2012). Briefly, 9 cm diameter filter paper discs (Whatman No. 4) were impregnated with five different concentrations (0.94, 1.26, 1.57, 2.89 and 2.2 mg/cm^2) of extract along with three replicates and placed on the glass petri plates (9 cm diameter). The solvent (acetone) was allowed to evaporate at room temperature. Twenty adults of *S. oryzae* and *C. maculatus* were introduced separately into the center of each petri dish and covered tightly with lid. Rice and lentils were added in petri dishes for food (not treated). Each experiment was conducted in triplicate. Two control groups (+ve) and (-ve) i.e., with or without acetone, respectively were run in parallel with the experiment. The number of dead and moribund insects was counted with 24 h intervals after treatment till 96 h. Knockdown effect was also determined at their respective doses. The inhibitory oviposition effect of treated extracts on *C. maculatus* (10 pair) was calculated by counting the number of eggs laid with the help of magnifying glass at 2.2 mg/cm^2 applied dose after 96 h.

Statistical analysis

Mortality in control was corrected using Abbot's formula (Abbott, 1925). Data were subjected to statistical analysis using SPSS 21® software. Probit® analysis (Finney, 1971) was applied to calculate the LC_{50} values at 95% confidence interval. Bonferroni test was applied to check the statistical difference ($P = 0.05$) on the oviposition inhibitory effect of extracts on the *C. maculatus* (SPSS version 21® Statistical Package for Social Sciences, USA).

RESULTS

LC_{50} values obtained in seaweeds treatment to *C. maculatus* and *S. oryzae* are presented in Tables I and II respectively indicate the toxic effects of red seaweeds. Mortality in the control group was found considerably below 5%. Probit® analysis showed that *C. maculatus* was more susceptible with high mortality than that of *S. oryzae*. Although, increasing concentrations and exposure duration decreases LC_{50} values. Detailed data is presented as supplementary data (Supplementary Tables S1 and S2).

In general, toxic effects of different botanicals from seaweeds were found least effective after 24 h exposure. *S. oryzae* and *C. maculatus* showed low susceptibility to different test extracts at lower applied doses. Meanwhile, the dichloromethane extract of *A. taxiformis* (DA) was significantly effective against *C. maculatus* LC_{50} 1.14 mg/cm^2 after 24 h with complete control i.e., 100% mortality at 2.2 mg/cm^2 obtained after 48 h treatment with LC_{50} 0.75 mg/cm^2 (Table I). HL and ML showed toxicity next to DA extract with LC_{50} 1.24 mg/cm^2 and LC_{50} 1.55 mg/cm^2 respectively after exposure of 24 h.

Low survival of the *C. maculatus* (mortality 99%) at the dose 2.2 mg/cm^2 was found after 96 h in the hexane and methanol extracts of *L. karachiana* i.e., HL (LC_{50} 0.62 mg/cm^2) and ML (LC_{50} 0.82 mg/cm^2) and in the methanol extract of *A. taxiformis* i.e., MA (91% ± 2.2) (LC_{50} 1.23 mg/cm^2) (Table I). Good results were also obtained with the HJ (LC_{50} 1.44 mg/cm^2) and DL (LC_{50} 1.83 mg/cm^2) with approximately 70% mortality achieved at 2.2 mg/cm^2 after 96 h (Table I).

Knockdown effects of DA and HL were noted for *C. maculatus* prior to the mortality with sever paralysis and jerking effects. These extracts were also effective in inhibiting the oviposition rate. Significantly lower numbers of eggs were laid by *C. maculatus* on grains (Fig. 2).

In contrast, *S. oryzae* was found tolerant at all the higher applied doses of dichloromethane, methanol, and hexane extracts after 24 h. However, after 48 h only DA achieved significantly higher mortality (88 ± 4.4%) at 2.2 mg/cm^2 and

Table I. Lethal concentration and 95% fiducial limits (FL) of extracts from red seaweeds against *C. maculatus* at various time after treatment (h).

Code *, **	Time (h)	LC_{50} (mg/cm^2)	95% FL	Slope^a ± S.E	$(x^2)^{bd}$	Sig^c
HL	24	1.24	1.13 - 1.33	3.86 ± 0.46	2.41	0.491
	48	0.95	0.79 - 1.07	3.35 ± 0.45	4.46	0.216
	72	0.76	NC	2.98 ± 0.51	17.7	0.001
	96	0.62	NC	3.20 ± 0.57	13.6	0.003
HA	24	> 2.2	-	-	-	-
	48	> 2.2	-	-	-	-
	72	> 2.2	1.92 - 4.50	3.98 ± 0.54	6.16	0.104
	96	2.01	1.82 - 2.35	2.99 ± 0.47	2.60	0.456
HG	24	> 2.2	-	-	-	-
	48	> 2.2	-	-	-	-
	72	2.16	2.11 - 2.24	17.5 ± 2.35	0.74	0.862
	96	2.13	2.08 - 2.20	17.7 ± 2.26	0.92	0.812
HJ	24	> 2.2	-	-	-	-
	48	2.07	1.89 - 2.39	3.42 ± 0.49	3.68	0.298
	72	1.82	1.68 - 2.03	3.34 ± 0.47	3.23	0.357
	96	1.44	1.33 - 1.56	3.45 ± 0.45	3.92	0.271
DL	24	> 2.2	-	-	-	-
	48	2.14	1.81 - 3.35	3.90 ± 0.51	5.45	0.141
	72	2.06	1.60 - 26.0	3.29 ± 0.48	10.8	0.013
	96	1.83	1.69 - 2.04	3.45 ± 0.47	3.31	0.346
DA	24	1.14	1.04 - 1.23	4.18 ± 0.48	2.83	0.418
	48	0.75	0.25 - 0.99	4.00 ± 0.59	5.87	0.118
	72	0.74	0.55 - 0.82	9.11 ± 2.25	2.04	0.563
	96	NC	-	-	-	-
DG	24	> 2.2	-	-	-	-
	48	> 2.2	-	-	-	-
	72	2.12	1.93 - 2.46	4.38 ± 0.54	20.1	0.001
	96	1.92	1.61 - 2.89	4.88 ± 0.53	10.1	0.017
DJ	24	> 2.2	-	-	-	-
	48	> 2.2	-	-	-	-
	72	2.00	1.94 - 2.09	10.5 ± 1.09	3.06	0.382
	96	1.90	1.64 - 2.48	9.00 ± 0.86	15.7	0.001
ML	24	1.55	1.35 - 1.80	6.37 ± 0.53	9.18	0.027
	48	1.28	0.63 - 1.67	5.68 ± 0.51	24.0	0.001
	72	1.11	0.04 - 1.45	4.78 ± 0.51	22.2	0.001
	96	0.82	0.50 - 1.00	3.68 ± 0.54	13.6	0.003
MA	24	1.73	1.27 - 5.22	3.49 ± 0.47	12.6	0.006
	48	1.48	1.17 - 1.82	4.77 ± 0.49	10.4	0.015
	72	1.32	1.24 - 1.39	5.14 ± 0.49	2.974	0.396
	96	1.23	1.14 - 1.30	4.71 ± 0.49	2.893	0.408
MG	24	> 2.2	-	-	-	-
	48	> 2.2	-	-	-	-
	72	2.16	1.87 - 2.87	2.17 ± 0.45	0.703	0.872
	96	1.94	1.64 - 2.80	1.60 ± 0.44	1.183	0.757

NC indicated that values not calculated by software. (-) indicated values not calculated found >2.2 mg/cm^2. ^{a}, Slope of the concentration dependent mortality regression line ± standard error; ^{b}, Chi square value; ^{c}, Significance level; ^{d}, df (3). *Codes are arranged as per extraction from different solvents; **H, D, and M in codes represents hexane, dichloromethane, and methanol extracts while J, L, A, and G represents *J. rubens*, *L. karachiana*, *A. taxiformis*, and *G. foliifera*; LC_{50} of the MJ was found >2.2 mg/cm^2 after 96 h.

(56 ± 2.9%) at 1.57 mg/cm^2 (LC_{50} 1.43 mg/cm^2) (Table II). Complete mortality obtained after 96 h at a dose of 2.2 mg/cm^2 (LC_{50} 0.93 mg/cm^2) (Table II). In addition to the DA, profound toxic effects against *S. oryzae* was also determined in the MA (LC_{50} 1.16 mg/cm^2) and in the HA (LC_{50} 1.27 mg/cm^2), HG (LC_{50} 1.32 mg/cm^2) and HL (LC_{50} 1.43 mg/cm^2) after 96 h (Table II). HJ also provided significant toxicity (LC_{50} 1.64 mg/cm^2) with >50% mortality achieved after 72 and 96 h exposure at higher applied doses (Table II).

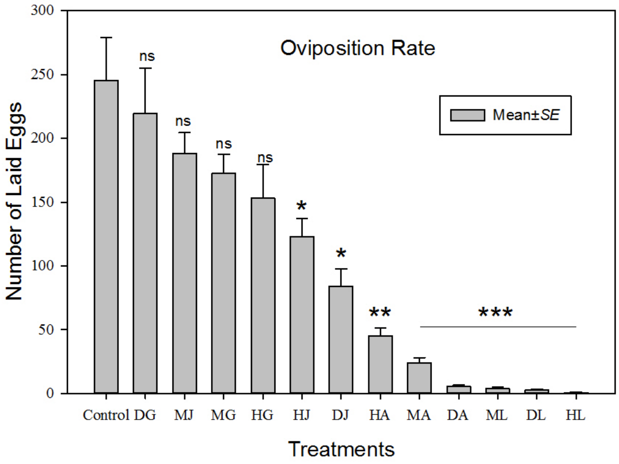

Fig. 2. Oviposition effects of red seaweeds on *C. maculatus*. Average proportion of 10 pairs of *C. maculatus* eggs laid on grains treated with four red seaweeds extracts (2.2 mg/cm^2) after 96 h exposure. Data is given in the mean percentage (n= 3) and their standard error. Asterisks on bars are significant levels at $P < 0.05$ (ns, non-significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). H, D, M in codes represent hexane, dichloromethane and methanol extracts while J, L, A and G in codes represent *J. rubens, L. karachiana, A. taxiformis*, and *G. foliifera*.

DISCUSSION

Pest damage in store commodities, including cash crops adversely affects their quality and quantity. Chemical pesticides are the common practice. Fumigants such as aluminum phosphide, sulfur dioxide, ethylene dichloride, methyl format, methyl bromide are widely used in storehouses. Some countries reported genetic resistance in insects against phosphine, an alarming situation (Afzal *et al*., 2019). In the current study red seaweeds have proven significant contact and fumigant effects against *S. oryzae* and *C. maculatus*. Results showed that botanicals of red seaweeds attribute towards strong activity against both the species tested. These active compounds should be isolated and identified. In general, red seaweeds are reported to be enriched with the terpenes, alkaloids and polyphenolic compounds (Perez *et al*., 2016).

Wide range of secondary halogenated metabolites has been isolated from seaweeds showed significant antifeedant and repellent effects (McClintock and Baker, 2001). However, isolation of bioactive compounds and toxicity assessment of the seaweeds inhabiting coast of Karachi (northern Arabian Sea) have not been carried out. Systematically, the only study tested the toxic effects of red, brown, and green seaweeds against the five species of stored grain pest i.e., *Callasobrocus analis, Rhyzopertha dominica, Sitophilus oryzae, Tribolium castaneum* and *Trogoderma granarium* by applying only one dose (200 mg) of one extract (methanol) in filter paper (Rizvi and Shameel, 2003). Authors reported that the red seaweed *Osmunda pinnatifida* was active on number of insects tested with 20% mortality while the brown seaweed *Jolyna laminariodies* exhibited moderate effects (with 40% mortality) after 24 h of application, rest of seaweeds showed no effects.

Results of the current study also supported these observations determining low mortality after 24 h of application. In the present study, both the species (*S. oryzae* and *C. maculatus*) showed low susceptibility to most of the extracts. However, Rizvi and Shameel (2003) did not determine the dose dependency and time related effects of seaweeds. Thus, current experiment encompassed and studied the contact or fumigant toxicity varies with insect species, extract's concentration and exposure time. The contact toxicity tests of the seaweed extracts against *S. oryzae* showed very low susceptibility in all treatments within 24 h. In such course of time no any extract achieved >40% mortality (LC_{50} >2.2 mg/cm^2) (Table II and Supplementary Table II). Maximum mortality (>90%) was achieved in most of the extracts at higher doses and/or exposing insects for longer duration of time (48-96 h) especially in the case of DA, MA and HL. The mortality was found decreasing in following order; HA, HG (>70%) and HJ and ML (>60%). *C. maculatus* was more susceptible towards the seaweed extracts with studied mortality. Knockdown effect was also noted prior to the mortality. Among four red seaweeds *A. taxiformis* showed significant toxic effects towards *C. maculatus* adults along with marked knockdown effect.

Exposure of *C. maculatus* with the DA (1.14 mg/cm^2) and MA (1.73 mg/cm^2) extracts of *A. taxiformis* induced 50% mortality after 24 h application. In general, treatment with *L. karachiana* extracts significantly reduce the *C. maculatus* population, while treating with *J. rubens* was not effective with the exception of hexane extract (HJ) inducing 50% mortality after 48 h (Table II). Fukuzawa and Masamune (1981) in their study also reported the toxic effect of two isolated acetylenic sesquiterpene compounds from red seaweed *Laurencia pinnata* on the bean beetle *Callosobruchus chinensis*. Similarly, the non-polar crude extract and isolated acetogenin(s) from *Laurencia papillosa* also exhibited high insecticidal activity against the 3rd instar larvae of *Tribolium confusum* (Abou-Elnaga *et al*., 2011).

Table II. Lethal Concentration and 95% fiducial limits (FL) of extracts from red seaweeds tested against *Sitophilus oryzae* at various time after treatment (h).

Code*, **	Time (h)	LC_{50} (mg/cm^2)	95% FL	Slope^a±S.E	$(x^2)^{bd}$	Sig^c
HL	48	1.81	1.63 - 2.08	10.2 ± 0.87	11.2	0.01
	72	1.54	1.02 - 2.55	4.56 ± 0.48	19.0	0.001
	96	1.43	0.69 - 2.25	5.04 ± 0.49	24.9	0.001
HA	48	> 2.2	-	-	-	-
	72	1.68	1.36 - 2.33	3.87 ± 0.48	8.47	0.037
	96	1.27	0.34 - 1.97	3.77 ± 0.46	15.0	0.002
HG	48	2.04	1.64 - 17.4	5.95 ± 0.65	20.5	0.001
	72	1.62	1.33 - 2.11	3.67 ± 0.46	6.84	0.077
	96	1.32	0.92 - 1.61	5.31 ± 0.49	14.7	0.002
HJ	48	2.19	2.07 - 2.35	7.56 ± 0.91	4.9	0.176
	72	1.93	1.80 - 2.09	4.58 ± 0.52	1.63	0.651
	96	1.64	1.54 - 1.76	4.23 ± 0.48	2.02	0.568
DA	48	1.43	1.36 - 1.51	5.35 ± 0.50	3.17	0.365
	72	1.17	1.01 - 1.29	7.72 ± 0.63	6.34	0.096
	96	0.93	0.85 - 0.99	7.08 ± 0.78	3.56	0.313
ML	48	> 2.2	-	-	-	-
	72	> 2.2	-	-	-	-
	96	1.91	1.80 - 2.06	5.12 ± 0.55	0.96	0.809
MA	48	1.71	1.57 - 1.90	3.66 ± 0.48	5.96	0.113
	72	1.30	1.23 - 1.37	5.56 ± 0.51	3.00	0.392
	96	1.16	0.88 - 1.35	4.94 ± 0.50	7.27	0.001
MG	48	> 2.2	-	-	-	-
	72	> 2.2	-	-	-	-
	96	> 2.2	1.94 - 4.93	3.62 ± 0.52	5.58	0.134

Columns values marked with "-" indicated that values not calculated. ^{a}, Slope of the concentration dependent mortality regression line ± standard error; ^{b}, Chi square value; ^{c}, Significance level; ^{d}, df (3). *Codes are arranged as per extracts from different solvents; **H, D, and M in codes represents hexane, dichloromethane, and methanol extracts while J, L, A, and G represents *J. rubens*, *L. karachiana*, *A. taxiformis*, and *G. foliifera*; DL, DG, and DJ were found least active (LC_{50} >2.2 mg/cm^2) and MJ showed no mortality after 96 h of treatment.

It was also observed that owing to the extractability some of the extracts were least active against stored grain pests. The polar solvent (methanol) extracts more constituents than a polar solvent (hexane). Dichloromethane has an intermediate polarity. *A. taxiformis* and *L. karachiana* possess potent toxic, knockdown and oviposition effect. The constituents present in applied botanicals derived from *A. taxiformis* using dichloromethane solvent exhibited knockdown effects, showing the presence of fumigant and/or contact toxicants in the extract which possibly inhibit the neurotransmitter or alter the insect physiological phenomenon. Volatile compounds enter the insect body through spiracle resulting in accumulation of CO_2 which in turn suffocates insects. Suffocation keeps spiracle open and more fumigants enter attach with hemoglobin causing extra cellular fluid and/or hemolymph acidosis (Sugiura *et al*., 2008).

Inhibition of neurotransmitters e.g. acetylcholinesterase (AChE) induces damages by blocking sodium-potassium channel and disturbing nerve-membrane potential effecting CNS and PNS (Rattan, 2010). Consequently, knockdown effect which was observed in the *C. maculatus* while exposing insects to the dichloromethane extract of *A. taxiformis* (DA) and hexane extract of *L. karachiana* (HL) induced high mortality within 24 h of application. In addition, the marked decline in egg laying by *C. maculatus* in these treated extracts was also determined (Fig. 2). Studied extracts from *L. karachiana* and *A. taxiformis* significantly reduced the number of eggs laid by *C. maculatus* ($P < 0.001$) subsequently reducing adult emergence. Current results are also in agreement with Schmidt *et al*. (1991) who reported the significant effects of vapours of *Acorus calamus* oil in adult mortality and in the declining the egg laying after 96 h of treatment.

Sahayaraj and Kalidas (2011) also studied the toxic effect of brown seaweed *Padina pavonica* against cotton sucking pest *Dysdercus cingulatus* (Fab) (Pyrrhocoridae). Chloroform and benzene extracts of *P. pavonica* induced high nymphal mortality, along with the reduction in the egg hatchability and abnormalities in embryonic development. The eggs were found varying in color, shape, and size. In the current study, all treatments of *A. taxiformis* and *L. karachiana* that significantly reduce the eggs laying by *C. maculatus* counted after 96 h of treatment ultimately reduces seed damage in turn become beneficial in making the grains viable for the human and animal consumption.

CONCLUSION

To conclude dichloromethane extract of *A. taxiformis* (DA) was found insecticidal. *C. maculatus* was being more susceptible. Hence, suggesting possible use in the further experiments and stored product pests control. Additional experiments are required to understand the mode of action of the particular compounds involved in the mortality, knockdown effect, reduced oviposition, and effects on the progeny, egg hatchability, adult emergence, and embryonic development in order to understand and to make the use of botanicals of red seaweeds at large scale in controlling pests in stored grain commodities.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjz/20..........

Statement of conflict of interest

The authors have declared no conflict of interest

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Supplementary Material

Insecticidal Potential of Botanicals from Red Seaweeds against Stored Grain Pests, Rice Weevil (*Sitophilus oryzae* L.) and Cowpea Weevil (*Callosobruchus maculatus* Fab.)

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Supplementary Table S1. Toxic effects of different extracts from red seaweeds against *Callosobrocus maculatus.*

Code*	Concen-trations	Mortality (mean % ± SE, n = 3)			
	(mg/mL)	24 h	48 h	72 h	96 h
HA	0.94	0±0^{a}	7.7±2.3^{a}	7.7±2.3^{a}	19±5.8^{a}
	1.26	0±0^{a}	10±1.1^{a}	16±2.3^{b}	26±2.9^{b}
	1.57	3±1.9^{a}	8.8±1.1^{a}	17±1.9^{b}	33±4.0^{b}
	1.89	10±3.3^{a}	14±2.9^{a}	33±3.9^{c}	45±2.9^{b}
	2.2	24±2.9^{b}	32±7.7^{b}	52±4.1^{d}	59±1.1^{c}
HG	0.94	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
	1.26	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
	1.57	0±0^{a}	0±0^{a}	0±0a	0±0^{a}
	1.89	3±1.9^{a}	9±1.1^{a}	16±2.9^{b}	18±4.0^{b}
	2.2	41±5.9^{b}	47±8.8^{b}	54±7.3^{c}	59±4.8^{c}
HL	0.94	33±3.3^{a}	50±5.7^{a}	60±11^{a}	70±5.7^{a}
	1.26	52±4^{b}	67±1.1^{ab}	81±4.8^{a}	86±3.9^{ab}
	1.57	59±4^{b}	72±4^{b}	76±3.3^{ab}	91±2.9^{b}
	1.89	77±3.3^{c}	80±5.7^{bc}	80±5.7^{ab}	86±3.8^{ab}
	2.2	86±2.9^{c}	94±1.9^{c}	98±1.1^{b}	99±6.7^{b}
HJ	0.94	0±0^{a}	13±5.1^{a}	19±4.8^{a}	30±1.9^{a}
	1.26	9±2.9^{a}	24±4.0^{ab}	29±3.6^{ab}	36±2.9^{a}
	1.57	11±2.9^{a}	29±1.1^{ab}	39±4.8^{ab}	56±4.9^{b}
	1.89	34±4.3^{b}	40±5.8^{b}	47±3.3^{bc}	62±6.2^{b}
	2.2	49±4.8^{c}	59±2.5^{c}	67±3.3^{c}	78±4.0^{b}
DA	0.94	38±6.7^{a}	67±8.8^{a}	81±7.3^{a}	100±0^{a}
	1.26	56±2.9^{b}	82±4.0^{b}	100±0^{b}	100±0^{a}
	1.57	68±1.1b^{c}	88±4.0^{bc}	100±0^{b}	100±0^{a}
	1.89	79±4.8^{cd}	91±1.1^{bc}	100±0^{b}	100±0^{a}
	2.2	92±2.2^{d}	100±0^{c}	100±0^{b}	100±0^{a}
DG	0.94	2±1.7^{a}	3±1.3^{a}	11±1.1^{a}	11±1.1^{a}
	1.26	10±3.4^{a}	10±3.4^{a}	11±1.1^{a}	16±2.9^{ab}
	1.57	10±2.5^{a}	10±2.5^{a}	13±3.3^{a}	24±3.9^{b}
	1.89	13±3.3^{a}	29±1.1^{b}	38±4.0^{b}	48±3.9^{c}
	2.2	24±2.9^{b}	42±4.0^{b}	60±1.9^{c}	70±1.3^{d}
DL	0.94	6±2.9^{a}	11±1.1^{a}	11±1.1^{a}	14±4.4^{a}
	1.26	7±3.3^{a}	16±2.9^{a}	32±4.0^{b}	34±4.4^{b}
	1.57	7±3.3^{a}	28±2.2^{b}	30±1.1^{b}	35±2.9^{b}
	1.89	14±2.9^{a}	36±2.9^{b}	36±2.9^{b}	50±1.9^{bc}
	2.2	34±2.9^{b}	59±2.9^{c}	61±2.9^{c}	63±3.3^{c}
DJ	0.94	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
	1.26	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
	1.57	5±2.9^{a}	16±3.3^{b}	16±3.3^{b}	34±4.4^{b}
	1.89	5±2.9^{a}	20±5.8^{b}	39±4.8^{c}	50±5.7^{bc}
	2.2	16±3.3^{b}	31±4.2^{b}	64±2.9^{d}	65±2.9^{c}
	1.26	9±2.9^{a}	24±4.0^{ab}	29±3.6^{ab}	36±2.9^{a}

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Code*	Concentrations	Mortality (mean % ± SE, n = 3)			
	(mg/mL)	24 h	48 h	72 h	96 h
	1.26	39±4.8^{b}	43±3.3^{b}	52±2.6^{b}	56±1.6^{b}
	1.57	52±4.0^{bc}	60±3.8^{b}	66±2.9^{bc}	68±4.0^{bc}
	1.89	57±3.4^{bc}	58±1.7^{b}	75±5.0^{cd}	77±6.7^{cd}
	2.2	56±2.9^{c}	82±4.0^{c}	88±4.0^{d}	91±2.2^{d}
MG	0.94	3±1.3^{a}	10±3.4^{a}	23±3.3^{a}	33±3.3^{a}
	1.26	0±0^{a}	11±1.1^{a}	29±1.1^{a}	36±2.9^{a}
	1.57	6±2.9^{ab}	14±4.4^{a}	36±2.9^{ab}	41±6.7^{ab}
	1.89	16±2.9^{b}	20±5.8^{a}	46±2.9^{bc}	50±1.9^{bc}
	2.2	36±2.9^{c}	38±1.1^{b}	52.2±1.1^{c}	56±2.9^{c}
ML	0.94	13±3.3^{a}	31±5.9^{a}	44±6.5^{a}	66±4.9^{a}
	1.26	22±6.2^{ab}	42±6.2^{ab}	57±6.4^{a}	70±3.6^{a}
	1.57	43±6.9^{b}	54±7.2^{b}	62±3.2^{a}	78±8.8^{ab}
	1.89	76±2.9^{c}	84±2.9^{c}	87±3.2^{b}	91±1.3^{bc}
	2.2	85±2.9^{c}	99±1.1^{c}	100±0^{b}	100±0^{c}
MJ	0.94	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
	1.26	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
	1.57	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
	1.89	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
	2.2	0±0^{a}	9±5.8^{a}	12±4.0^{b}	19±5.8^{a}
Acetone (+ control)		0±0	0±0	3.3 ± 3.3	5±2.9
(- control)		0±0	0±0	0±0	3.3± 3.3

*H, D, and M in codes represents hexane, dichloromethane, and methanol extracts while J, L, A, and G represents *J. rubens*, *L. karachiana*, *A. taxiformis*, and *G. foliifera*. Superscript ^{a} to ^{d} indicate significant difference among variables (Bonferroni text; P < 0.05).

Supplementary Table S2. Toxic effects of different extracts from red seaweeds against *S. oryzae.*

Codes*	Concentrations	Mortality (mean % ± SE, n = 3)			
	(mg/mL)	24 h	48 h	72 h	96 h
HA	0.94	3±0.7^{a}	10±2.9^{a}	10±2.9^{a}	22±4.4^{a}
	1.26	3±0.3^{a}	17±3.3^{ab}	39±4.8^{b}	64±6.7^{b}
	1.57	9±1.1^{ab}	22±6.2^{ab}	52±1.7^{b}	61±4.9b
	1.89	15±7.6^{ab}	25±2.9^{ab}	57±8.8^{b}	76±4.5^{b}
	2.2	25±2.9^{b}	35±5.2^{b}	62±6.2^{b}	77±3.3^{b}
HG	0.94	0±0^{a}	0±0^{a}	21±5.9^{a}	29±1.1^{a}
	1.26	2±2^{ab}	6±2.9^{a}	22±6.2^{a}	30±2.9^{a}
	1.57	5±2.9^{abc}	41±2.9^{b}	56±2.9^{b}	72±6.0b
	1.89	10±1.9^{bc}	41±4.9b	62±4.3^{b}	81±3.4^{b}
	2.2	14±2.9^{c}	50±1.9^{b}	65±2.9^{b}	89±4.8^{b}
HL	0.94	0±0^{a}	1±0^{a}	23±8.8^{a}	27±6.7^{a}
	1.26	0±0^{a}	7±1.7^{a}	30±5.8^{a}	30±5.8^{ab}
	1.57	0±0^{a}	20±5.8^{a}	42±6.0^{a}	50±3.3^{b}
	1.89	10±5.8^{a}	52±6.0^{b}	58±10^{ab}	65±5^{b}
	2.2	35±2.9^{b}	87±8.8^{c}	88±9.3^{b}	95±2.9^{c}
HJ	0.94	0±0^{a}	0±0^{a}	9±1.1^{a}	13.3±1.9^{a}
	1.26	0±0^{a}	2±0^{a}	17±1.9^{b}	31±2.9^{b}
	1.57	7±1.9^{a}	14±2.9^{b}	33±3.8^{b}	52±6.2^{c}
	1.89	33±3.9^{b}	39±1.1^{c}	52±4.0^{c}	60±1.9^{c}
	2.2	36±2.9^{b}	44±3.0^{c}	58±4.4^{c}	67±1.5^{c}
DA	0.94	2±0.7^{a}	17±4.4^{a}	28±4.4^{a}	53±8.8^{a}
	1.26	8±0.7^{a}	40±5.8^{b}	52±7.3^{b}	80±0^{b}
	1.57	9±2.4^{ab}	56±2.9b^{c}	82±6.2^{c}	92±4.1^{b}
	1.89	26±2.9^{b}	69±6.8^{c}	96±2.9^{c}	100±0^{b}
	2.2	30±5.0^{b}	88±4.4^{c}	100±0^{c}	100±0^{b}
DG	0.94	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
	1.26	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
	1.57	0±0^{a}	3±1.9^{a}	6±2.9^{a}	7±1.9^{b}
	1.89	3±1.9^{a}	6±2.9^{b}	26±2.9^{b}	28±4^{c}
	2.2	16±2.9^{b}	16±2.9^{b}	34±3.1^{c}	36±2.9^{c}
DL	0.94	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
	1.26	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
	1.57	0±0^{a}	0±0^{a}	8±1.7^{a}	12±4.4^{b}
	1.89	0±0^{a}	5±2.8^{ab}	10±5.7^{a}	36±2.01^{c}
	2.2	0±0^{a}	11.6±4.4^{b}	30±5.7^{b}	40±2.7^{c}
DJ	0.94	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
	1.26	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}
	1.57	0±0^{a}	0±0^{a}	0±0^{a}	2±0.7^{a}
	1.89	0±0^{a}	0±0^{a}	0±0^{a}	2±0.7^{a}

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Codes*	**Concen-trations**	**Mortality (mean % ± SE, n = 3)**			
	(mg/mL)	**24 h**	**48 h**	**72 h**	**96 h**
	2.2	0±0^{a}	3±1.9^{a}	6±2.9^{a}	10±1.9^{a}
MA	0.94	0±0^{a}	12±1.7^{a}	20±1.6^{a}	36±2.9^{a}
	1.26	4±2.2^{a}	32±6.2^{b}	52±6.2^{b}	57±1.9^{b}
	1.57	12±2.2^{a}	51±4.9^{b}	62±6.0^{b}	64±2.9^{b}
	1.89	20±1.93^{bc}	49±2.6^{cd}	81±2.9^{c}	85±2.9^{c}
	2.2	37±1.93^{c}	61±2.9^{d}	91±2.9^{c}	96±2.9^{c}
MG	0.94	0±0^{a}	0±0^{a}	5±1.1^{a}	11±3.4^{a}
	1.26	0±0^{a}	0±0^{a}	9±1.1^{a}	13±3.3^{a}
	1.57	0±0^{a}	8±2.2^{a}	11±1.1^{a}	19±5.8^{ab}
	1.89	8.8±1.1^{b}	11±1.1^{ab}	18±1.1^{b}	36±2.9^{b}
	2.2	17±1.9^{c}	37±3.8^{b}	44±2.9^{c}	49±1.9^{b}
ML	0.94	0±0^{a}	0±0^{a}	5±0^{a}	5±0^{a}
	1.26	0±0^{a}	3±3^{a}	10±2.9^{a}	18±1.7^{a}
	1.57	0±0^{a}	3±3^{a}	12±1.7^{a}	35±2.9^{b}
	1.89	0±0^{a}	5±2.9^{a}	15±2.9^{a}	45±2.9^{b}
	2.2	15±5.0^{b}	22±1.7^{b}	35±2.9^{b}	63±6.0^{c}
Acetone (+ control)		0±0	5±2.9	5±2.9	5±2.9
(- control)		0±0	0±0	3.3 ± 3.3	3.3 ± 3.3

*H, D, and M in codes represents hexane, dichloromethane, and methanol extracts while J, L, A, and G represents *J. rubens*, *L. karachiana*, *A. taxiformis*, and *G. foliifera*. Superscript ^{a} to ^{d} indicate significant difference among variables (Bonferroni text; $P < 0.05$). No mortality observed in "MJ" in any applied concentration.