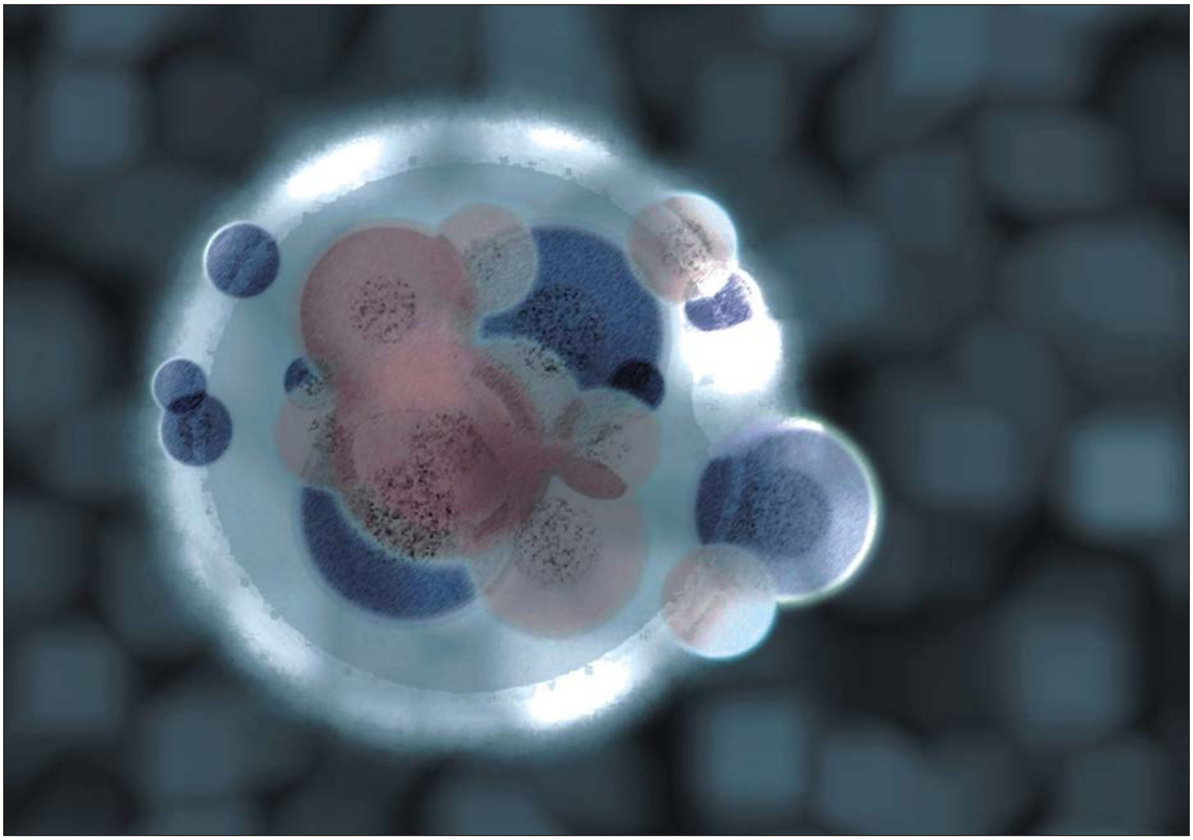

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Insecticidal Bustle of Corallivorous Gastropod, *Drupa margariticola*

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Abstract: The aim of this study, the coral reef associated mollusc *Drupa margariticola* was screened for their insecticidal activity against a dreadful rice pest *Sitophilus oryzae,* The insecticidal activity of the crude and partially purified extracts of *D. margariticola* was tested using contact bioassay by the modified method. The extracts of acetone, ethyl acetate and dichloromethane caused 100% (10), 80.67% (8.67±1.25) and 80.0% (8±0.82) mortality respectively against *S. oryzae* at a concentration of 50 mg mL^{-1}. The 100% acetone column purified fractions of extracts was found cause 100% at a concentration of 20 mg. The ED_{50} value of crude and column purified acetone extracts of *D. margariticola* was found to be lower 21.08 and 4.09 mg mL^{-1}, respectively. The extract of *D. margariticola* screened for insecticidal activity clearly shows that the extract of acetone may possess some biologically active compounds. The exact route of entry is doubtful, which, in future can be cleared with studies on the mode of action of the extract tested.

Key words: Mollusc, *Drupa margariticola*, insecticidal activity, *Sitophilus oryzae*

INTRODUCTION

Insects have tremendous economic importance and are frequently studied under the domains of Zoology, Agriculture, Medical entomology and Parasitology. Insects have been controlled with synthetic insecticides in the last fifty years. Most insecticidal compounds fall within four main classes, such as organochlorides, organophosphates, the carbamates and pyrethroids. Out of these, the major classes in use today are organoclorides and carbamates (Dorow, 1993; Ware, 1982). Many synthetic pesticides are halogenated compounds. Since, halogenation is a natural process in marine plants, the compounds produced are likely to possess agrichemical activity. Since, these chemicals are toxic and biologically active in their use for insect control has become a serious concern (Balogh and Anderson, 1992).

The repeated use of pesticides for the control of pests has raised the problem of contamination of soil, atmosphere and water etc. The contamination of pesticides can be quantified either by bioassay or chemo-assay. The term bioassay means the measurement of intensity of physical, chemical and biological stimulus on the living organisms. For the bioassay of insecticides, mostly insects are used as a tool to estimate the small quantities of insecticides and insecticidal residues, although organisms like protozoans, crustaceans and fishes are also used. In the field, the bioassay technique of insecticides and pesticides are used to evaluate toxicity of insecticide and insecticide residues and these breakdown products.

To date, study focused on isolating insecticidal leads from marine origin has resulted in the report of about 40 active compounds. Insecticidal compounds were isolated from red algae (Watanabe *et al.*, 1989), corals (Chellaram *et al.*, 2005; Grode *et al.*, 1983). Of the marine organisms, in particular, the molluscs have always proven to be potential sources of marine natural products with vast array of diversified bio-activities. There are massive evidences to witness marine molluscs as potential supply of potent metabolites (Hubert *et al.*, 1996; Chellaram and Edward, 2009). In the present study, the crude as well as partial purified extracts of the gastropods, *Drupa margariticola* associated to corals was screened for insecticidal activity against a dreadful rice pest *Sitophilus oryzae*.

MATERIALS AND METHODS

Study area: The Molluscan samples were collected by hand picking using SCUBA diving from the intertidal area

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at a depth of 5-7 m in Tuticorin coastal waters (Lat 8°45 and Long 78°13'E) of Southeast coast of India.

Extraction: Live *Drupa margariticola* associated to coral reef were collected from a depth of 5-6 m using SCUBA diving and were brought to the laboratory in live condition. The shells were opened and the soft body was taken out, cut into small pieces and air-dried to remove the water content. The extracts of the air-dried animals were obtained using different solvents such as acetone, ethyl acetate, dichloromethane, chloroform and methanol after cold steeping at -18°C. The extracts from each solvent were filtered separately for three times using Whatmann No.1 filter paper. The filtrate was poured in previously weighed Petri plates, evaporated to dryness and used for all the experiments.

Column purification of the active crude extracts: Partial purification of the crude extracts was carried out by Wright (1998) method. After initial screening, the higher activity was shown by acetone extract and it was fractionated by normal phase silica gel column chromatography by employing a step gradient solvent system from low to high polarity. Sequence of 100% hexane; 20% acetone: 80% Hexane, 40% acetone: 60% Hexane, 60% acetone: 40% hexane, 80% acetone: 20% hexane, 100% acetone, 80% acetone: 20% methanol, 60% acetone: 40% methanol, 40% acetone: 60% methanol, 20% acetone: 80% methanol and 100% methanol was used for elution. Each fraction thus obtained was once again evaporated, concentrated and assayed for insecticidal activity.

Features of rice pest *Sitophilus oryzae* (Coleoptera: Curculionidae): The rice weevil is small, 1/10 inch (2 to 3 mm) and stout in appearance. It is very similar in appearance to the granary weevil. However, the rice weevil is reddish-brown to black in color with four light yellow or reddish spots on the corners of the elytra (the hard protective forewings). The snout is long (1 mm), almost 1/3 of the total length. The head with snout is as long as the prothorax or the elytra. The prothorax (the body region behind the head) is strongly pitted and the elytra have rows of pits within longitudinal grooves. The larva is legless and stays inside the hollowed grain kernel. It is fat with a cream colored body and dark head capsule.

The rice weevil is one of the most serious pests in stored grains worldwide. This pest was grain originated in India and has been spread worldwide. Both the adults and larvae feed on whole grains. They attack wheat, corn, oats, rye, barley, sorghum, buckwheat, dried beans, cashew nuts, wild bird seed and cereal products especially macaroni. The adult rice weevil can fly and is attracted to lights.

Insecticidal activity of crude extracts: The insecticidal activity of the extracts of *D. margariticola* from different solvents such as acetone, ethyl acetate, dichloromethane, chloroform and methanol were tested using contact bioassay by the modified method (Broussalis *et al.*, 1999). A 100 mg of each of the dried extracts was dissolved in 1 mL of their respective solvents and from these 500, 250, 100, 50, 25 and 10 μL were poured in separate Petri plates in triplicates and allowed to evaporate overnight to get the same concentration in mg mL^{-1} of extract. Controls with solvents alone were taken in separate Petri plates and allowed to evaporate overnight. Ten healthy adults of *Sitophilus oryzae* were introduced into each petri dish and sufficient food was provided to the test organisms so that the death due to starvation could be ruled out. After 24 h, the number of dead insects was counted and percentage of mortality was noted. The efficiency of the extracts obtained with different solvents at varied concentrations in killing the insects was determined.

Insecticidal activity of column purified extracts: Column purified fractions of the extracts was carried out by Wright (1998) method. After initial screening, the extract showing activity obtained with acetone was fractionated using normal phase silica gel column chromatography, employing a step gradient solvent system from low to high polarity. After step gradient, the fractions thus obtained were once again evaporated and concentrations of 20, 10, 5 0.25 and 0.1 mg mL^{-1} of the extracts were poured and tested for insecticidal activity. Number of the dead insects was counted after 24 h and the percentage of mortality was once again noted. The LC_{50} values of the both crude and column purified fractions were determined by the probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

Insecticidal potential of the crude extracts of gastropods: The insecticidal activity of the crude extracts of *Drupa margariticola* obtained using different solvents was tested against *Sitophilus oryzae* and the percentage mortality is given in Table 1. All the solvent's extracts were found to cause mortality, but 100% (10±0) mortality was observed only for the insects exposed to the extracts of acetone at a concentration of 50 mg after 24 h. The extracts by ethyl acetate and dichloromethane caused only 80.67% (8.67±1.25) and 80.0% (8±0.82) mortality respectively at the same concentration.

Insecticidal potential of the column purified fractions of gastropod: The 100% acetone column-purified fractions of *D. margariticola* were found cause 100% at a concentration of 20 mg mL^{-1} against *S. oryzae*, where as,

Table 1: Insecticidal activity of the crude extracts of *Drupa margariticola* against *Sitophilus oryzae*

	No.of insects died				
Conc. of the extracts (mg)	EA	A	DCM	CHL	M
50	8.67±1.25	10	8.00±0.82	7.33±0.94	8.00±0.82
25	6.67±1.25	8.33±0.47	6.33±0.94	6	5.67±1.24
10	4.67±0.47	5.33±0.94	4.33±0.47	4	3.67±0.94
5	3.33±0.94	4.33±0.94	3.33±0.47	3.33±0.47	3.33±0.47
0.25	2.67±1.25	3.67±0.47	2.67±0.47	2.33±0.47	2.33±0.47
0.1	1.67±0.47	2.33±0.47	1.00±0.81	1.33±0.47	1.33±0.47
Control	0	0	0	0	0

A: Acetone, EA: Ethyl acetate, DCM: Dichloromethane, CHL: Chloroform and M: Methanol. Data are expressed as average of 3 values±SD

Table 2: Insecticidal activity of the column purified extracts of *Drupa margariticola* against *Sitophilus oryzae*

	Percentage of mortality to insects								
Conc. of the extracts (mg)	H-60: A-40	H-40: A-60	H-20: A-80	A	A-80: M-20	A-60: M-40	A-40: M-60	A-20: M-80	M
20	46.7±0.6	63.3±0.6	93.3±0.6	100	93.3±0.6	80.0±1.0	70.0±1.0	60.0±1.0	43.3±0.6
10	30.0±0.0	36.7±0.6	76.7±0.6	83.3±0.6	76.7±0.6	56.7±0.6	50.0±1.0	46.7±1.16	23.3±0.6
5	6.7±0.6	16.7±0.6	23.3±0.6	60.0±1.0	63.3±0.6	53.3±0.6	23.3±1.5	30.0±1.0	10.0±1.0
0.25	6.7±0.6	13.3±0.6	46.7±0.6	53.0±0.58	33.3±0.6	20.0±1.0	20.0±0.0	16.7±0.6	6.7±0.6
0.1	3.3±0.6	6.7±0.6	30.0±1.0	33.3±0.6	16.7±0.6	13.3±0.6	10.0±0.0	6.7±1.6	3.3±0.6
0.05	0	3.3±0.6	13.3±0.6	16.7±0.6	10.0±1	6.7±0.6	6.7±0.6	3.3±0.6	0
Control	0	0	0	0	0	0	0	0	0

H: Hexane, A: Acetone and M: Methanol

Table 3: Crude extracts for ED_{50} values (mg)

Extracts	Values
Ethanol	38.0
Acetone	21.08
Chloro form	24.5
Dichloromethane	28.6
Methanol	32.0

Table 4: Column purified fractions of ED_{50} values (mg)

Column purified fractions	Value
H: A 80:20	48.45
H: A 60:40	38.15
H: A 40:60	7.90
H: A 20:80	5.49
A	4.09
A: M 80:20	11.80
A: M 60:40	15.30
A: M 40:60	23.70
A: M 20:80	43.50
ME	51.60

A: Acetone, ME: Methanol and H: Hexane

column purified extracts of the *D. margariticola,* 60:40 hexane: acetone and 60:40 methanol and acetone fractions were found to cause 46.7 and 43.3% mortalities respectively at the same concentration (Table 2). Significance in the efficacy as well as concentration of extracts such as ethyl acetate and dichloromethane, acetone and ethyl acetate and acetone and dichloromethane in killing the insects were analyzed. There was not much significant difference between the extracts obtained from different solvents and the concentration as well for organisms *D. margariticola*.

1.ED_{50} values for the crude and column-purified fractions of the gastropods: The ED_{50} values for the extracts ethyl acetate, chloroform, dichloromethane methanol of *D. margariticola* were found to be 38, 24.5, 28.6 and 32 mg mL^{-1}, respectively. The ED_{50} value of acetone extract of *D. margariticola* was found to be lower 21.08, which reveals that it is efficient in killing the insect than the other four solvents (Table 3). It was also observed that the ED_{50} value of 100% acetone fractions of *D. margariticola* was noted to be 4.09 mg mL^{-1} (Table 4), which is very much lower when compared with other fractions of the column purified extracts.

Rice weevil, *Sitophilus oryzae* is a common pest, which destroys stored rice, wheat and sorghum grains. A single larva is *S. oryzae* is reported to consume 10 mg of grain during its development. An estimated one third of a typical crop is lost due to pests and diseases and these are not systematically controlled (Melnikov, 1971). Much of the increase in agricultural productivity over the past half century has been due to the control of these pests with Synthetic Chemical Pesticides (SCPs) (Duke *et al*., 1993). Crop protection chemicals continue to be the major tool for protecting food and fiber crops from damaging pests. In 1990, the world market value of pesticides totaled nearly $23 billion, 28% of which was for insecticides (Melnikov, 1971). There are however, several reasons to search for alternative compounds to the SCPs for controlling pests.

In the present study, highest mortality was caused to *S. oryzae* by the acetone extracts of *D. margariticola*. However, that ethyl acetate extract of a sea weed, *Gracilaria crassa* caused 100% mortality to *S. oryzae*. The acetone extract of the winged oyster, *Pteria chinensis* caused 100% mortality to *Sitophilus oryzae* at a concentration of 20 mg (Chellaram *et al*., 2005).

Many novel insecticidal principles including, halichondramide, isolated from the sponge of the genus, *Halichondria* showed significant insecticidal activity at

25 mg mL^{-1}, especially against tobacco bud worm (Kernan and Faulkner, 1987). However, in an earlier work, the butanol fractions of the deep sea mollusc, *Nassarius suturalis* were found to 100% mortality against at a concentration of 200 mg.

The highly oxidized group of the insecticide briarances is already isolated from gorgonians, soft corals and sea pens (Grode *et al*., 1983). In the present study, the 100% column purified acetone fraction was observed to have lower ED_{50} values such as 4.09 mg mL^{-1}. This result suggests that the acetone extracts of the *D. margariticola* was proficient enough to cause mortalities to the test organisms even at low concentrations. In the same manner, the 100% column purified acetone extracts of *Turbo intercostalis* and *Cerithidea cingulata* showed still lower LC_{50} values (29.11 and 38.16 mg, respectively) (Gnanambal *et al*., 2005). Both crude and column purified fractions of the gastropod, *Drupa maragariticola* possess assured insecticidal ethics with lower ED_{50} value and it might be assumed that extracts contain insecticidal compounds.

CONCLUSION

The results indicate that the crude and column fractions (100% acetone 20:80 methanol: acetone and 80:20 acetone: hexane) of *D. margariticola* screened for insecticides activity and extracts of 100% acetone may possess some biologically active compounds. The activity of the column purified fractions was found to be prominent in comparison to the crude counterparts. So, the partially purified fractions of 100% acetone from *D. margariticola* undeniably provide a tool for the development of novel insecticides.

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