ISSN : 0974 - 7508 Volume 7 Issue 4

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 7(4), 2011 [187-190]

Insecticidal effect of oyster mushroom (*Pleurotus ostreatus*) against *Tribolium castaneum* (Herbst)

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Received: 25th June, 2011 ; Accepted: 25th July, 2011

ABSTRACT

This study scientifically examined the residual effect of fruiting body of oyster mushroom, *Pleuotus ostreatus*, against adult red flour beetle *Tribolium castaneum* (Herbst) at different time intervals. In residual film toxicity, hot water and methanol-chloroform extracts of oyster mushroom and petroleum ether and residual fractions of methanol-chloroform extract showed moderate activity against *Tribolium castaneum*. Residual fraction showed the lowest LD_{50} values (0.206 mg/cm^2) indicating its potent efficacy against *Tribolium castaneum*. The order of toxicity on *Tribolium castaneum* was residual fraction>petroleum ether fraction>methanol-chloroform extract>hot water. In addition, the results of this study also indicate that, the toxicity for each test sample against *Tribolium castaneum* was increased with increasing of time of exposure.

KEYWORDS

Oyster mushroom;
Pleuotus ostreatus;
Tribolium castaneum;
Residual flim.

INTRODUCTION

Pest control is a major issue for underdeveloped agricultural countries. Among the important stored-product insect pests, the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is common and most destructive pest throughout the world. This pest has been reported to attack the germ part (embryo portion) of the grain. Their presence in stored foods directly affects both the quantity and quality of the commodity[1]. Control of this pest relies heavily on the use of synthetic insecticides and fumigants, which has led to environmental pollution, increasing costs of application, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms in addition to direct toxicity to users[2-3]. Thus insecticides of natural origin are rational alternatives to synthetic insecticides. Oyster mushroom (*Pleuotus ostreatus*) is one of the popular cultivated edible mushrooms with high nutritional value and excellent medicinal properties[4]. Numerous articles and monographs contain detailed information on the physiology, chemistry, pharmacology and medicinal value of oyster mushroom[5-8]. The present research was under taken to investigate

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the insecticidal effect of hot water and methanol-chloroform extracts of oyster mushroom and petroleum ether and residual fractions of methanol-chloroform extract against adults *Tribolium castaneum*.

MATERIALS AND METHODS

Pleurotus ostreatus

Fresh fruiting bodies of oyster mushroom (*Pleurotus ostreatus*) were collected from the mushroom production center, Chapainawabganj, Bangladesh and identified at the Department of Botany, University of Rajshahi. A voucher specimen (BDRU-285) was deposited at the Department of Botany, University of Rajshahi, Bangladesh.

Preparation of extracts and fractions

The fresh fruiting bodies of *Pleurotus ostreatus* were sun dried for 7 days and finally in an electrical oven below 40°C for 48 hours to remove moisture completely. Then the dried fruiting bodies were pulverized to powder by a grinder machine. The powdered materials were weighed and placed in two different air-tight bottles to which hot water and methanol-chloroform (3:2) mixture was added. The contents were pressed through the markin cloth to get maximum amount of extract and filtration was done by Whatman filter paper No. 41 at 5 hrs and 15 days interval for extraction with hot water and methanol-chloroform mixture, respectively. Both extracts were concentrated with a rotary evaporator under reduced pressure at 60°C and finally 6.5 g of hot water extract and 16.5 g methanol-chloroform extract were obtained. Ten g methanol-chloroform extract was fractionated into 4.2 g petroleum-ether fraction by solvent-solvent partitioning[9]. After fractionation with petroleum ether, the remaining portion was evaporated with a rotary evaporator and 4.95 g extract was obtained as residual fraction. The output extracts and fractions were collected to glass vials and preserved in a refrigerator at 4°C.

Insects

Red flour beetle *Tribolium castaneum* were used to examine the pesticidal activity of oyster mushroom (*Pleuotus ostreatus*). Adult and larval stages of insect were taken from the Department of Zoology, University of Rajshahi, where pest culture maintained for last 10 years in an incubator at 30±1°C, 65% relative humidity and 12:12 hrs dark/light photoperiod which has been reported an optimum for rapid growth[10]. Insects were reared on a diet mixture of whole meal flour with Bakers yeast (19:1) in a Jar[11]. After every three days the medium was replaced by a fresh one to avoid conditioning by the larvae[12].

Residual film method of toxicity

Residual film method as described by Busvine[13], was used. A preliminary screening of different doses was performed on adults *Tribolium castaneum* to obtain 0% to 100% mortalities. For each extracts and fractions, 200 mg, 100 mg, 50 mg and 25 mg were dissolved separately in 5 ml of corresponding solvent to get concentrations of 40 mg/ml, 20 mg/ml, 10 mg/ml and 5 mg/ml, respectively, which were used as stock solutions. One ml of various concentrations for each sample was applied on petridishes (7 cm diameter) in such a way that it made a uniform film over the petridishes. For solvent evaporation, the petridishes were air dried leaving the extract on it. The actual extract present in 1 ml mixture was calculated and the dose per square centimeter was determined by dividing the value present in one ml with the area of the petridish. So, calculated doses were 1.2385, 0.6192, 0.3096 and 0.1548 mg/cm^2. After drying, 10 beetles were released in each petridish with three replications. A control batch was also maintained with the same number of insects after preparing the petridish by applying and evaporating the solvent only. The treated beetles were placed in an incubator at the same temperature as reared in stock cultures and the mortality of the beetles were counted after 6, 12, 24 and 30 hours post-exposure[14].

Statistical analysis

The mortality data were subjected to Probit analysis for the determination of LD_{50} values using the computer software SPSS of 14 version. Results with $p<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

In the present investigation, the toxicity of fruiting bodies of oyster mushroom (*Pleurotus ostreatus*) was

tested against adults of *Tribolium castaneum*. The mortality (%) were recorded and statistical data regarding LD_{50}, 95% confidence limit and chi-square values were calculated and presented in TABLE 1. The residual film toxicity showed that hot water and methanol-chloroform extracts and petroleum ether and residual fractions of methanol-chloroform extract of oyster mushroom (*Pleurotus ostreatus*) were found to be toxic to *Tribolium castaneum* (TABLE 1). The LD_{50} values for residual fraction against adult *Tribolium castaneum* were found to be 0.425, 0.396, 0.247 and 0.206 mg/cm^2 whereas they were 1.251, 1.334, 1.184 and 0.958 mg/cm^2 for hot water extract after 6, 12, 24 and 30 hrs of exposure, respectively. Methanol-chloroform extract showed LD_{50} values of 1.413, 1.264, 1.102 and 0.781 mg/cm^2 and in case of petroleum-ether fraction it were 0.647, 1.541, 0.241 and 0.194 mg/cm^2 for 6, 12, 24 and 30 hrs intervals, respectively. Depending on the LD_{50} values, it was found that among the test materials residual fraction showed the highest toxicity and hot water extract was less toxic on *Tribolium castaneum* insects. On the basis of the intensity of toxicity test materials were considered as the following order:

Residual extract> Petroleum-ether extract> Methanol-chloroform extract > Hot water extract.

The result demonstrates that toxicity of the extracts and fractions of oyster mushroom (*Pleurotus ostreatus*) were increased with the increasing of exposure time. This may clearly support that exposure time play an important role in influencing susceptibility. The present investigation is more or less similar to the findings of Talukder and Upadhyay who reported the insecticidal properties of neem oil, Pithraj (*Aphanamixis polystachya) and Piper nigrum* against *Tribolium castaneum*[15-16]. Our findings clearly reveal that oyster mushroom (*Pleurotus ostreatus*) is effective against *Tribolium castaneum* infestation and it can work as a promising natural pesticide.

ACKNOWLEDGEMENT

The authors wish to express their sincere thanks to the chairman, Department of Biochemistry and Molecular Biology, University of Rajshahi, for providing necessary laboratory facilities. The authors also would like to extend their grateful thanks to the Chairman, Department of Zoology, University of Rajshahi, for pro-

TABLE 1 : Dose mortality effect of oyster mushroom (*Pleurotus ostreatus)* extracts against *Tribolium castaneum* adults after 6, 12, 24 and 30 h of exposure.

Sample	Exposure time (Hours)	LD_{50} value (mg/cm^2)	95% confidence limits		Chi-squre (χ^2)
			Upper	Lower	
Hot water extract	6	1.251	3.175	1.002	0.271
	12	1.334	1.966	0.905	0.173
	24	1.184	1.502	0.783	2.220
	30	0.958	1.151	0.639	3.328
Methanol-Chloroform Extract	6	1.413	2.534	0.788	0.179
	12	1.264	2.096	0.762	0.377
	24	1.102	1.750	0.694	0.941
	30	0.781	1.117	0.545	0.481
Petroleum-ether fraction	6	0.647	0.893	0.468	1.686
	12	1.541	0.460	0.262	5.073
	24	0.241	0.306	0.188	6.122
	30	0.234	0.251	0.149	4.101
Residual fraction	6	0.425	0.654	0.275	6.958
	12	0.396	0.504	0.314	3.521
	24	0.247	0.312	0.194	1.437
	30	0.206	0.259	0.163	0.587

 # Values were based on four doses with 30 insects each. # Control groups showed no mortality. *Significant at P<0.05 level.

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viding required laboratory facilities to perform this experiment.

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