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LARVICIDAL AND INSECTICIDAL EFFECTS OF DIFFERENT EXTRACTS OF *MALLOTUS REPANDUS* (WILLD.) MUELL.- ARG. LEAF AND STEM AGAINST *CULEX QUINQUEFASCIATUS* SAY (DIPTERA: CULICIDAE) AND *SITOPHILUS ORYZAE* LINN. (COLEOPTERA: CURCULIONIDAE)

Md. Rakib Hasan[1], Nizam Uddin[1*], Md. Mahadi Hasan[1], Md. Monir Hossain[1], Mohammad Mostafa Kamal[1], Kaniz Fatema[1], H. M. Lutfor Rahman Mazumder[1], Kabirul Bashar[2], Md. Sohel Rana[1]

[1]Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh
[2]Department of Zoology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

*** Corresponding author email:** sami.pharm22@gmail.com

ABSTRACT

This study was designed to evaluate larvicidal bioassay of different extracts of *Mallotus repandus* against *Culex quinquefasciatus* and insecticidal bioassay against *Sitophilus oryzae* in the laboratory. In larvicidal bioassay, methanol extract of leaf, petroleum ether extract of leaf and methanol extract of stem showed good LC_{50} values (median lethal time) found in three consecutive days (24, 48 and 72 hours). Methanol extract of leaf showed the highest larvicidal activity in each specific time interval. Moreover, the extract also exhibited dose dependent lethal time effect (LT_{50}). In insecticidal bioassay, among all extracts ethyl acetate extract of stem showed the highest insecticidal activity after 24 and 48 hours while petroleum ether extract of leaf exhibited the highest insecticidal activity in 72 hours. Ethyl acetate extract of stem also showed dose dependent LT_{50}. Highly significant ($P < 0.0001$) relationship between different hours and LC_{50} values were found in both bioassays. These findings suggest that the extracts have potential toxicity against both insects.

Keywords: Larvicidal activity; Insecticidal activity; *Mallotus repandus*; *Sitophilus oryzae*; *Culex quinquefasciatus*; Malathion; Deltamethrin

INTRODUCTION

Mosquitoes are the most important public health concern which not only cause nuisance to humans but also transmit several diseases such as: filaria, Japanese encephalitis, malaria, dengue fever, chikungunya [1] and yellow fever [2]. These diseases largely diminish the health and quality of life of millions of people in subtropical and tropical countries [3]. Mosquito bites also cause skin allergy with inflammation and as a result irritation becomes persistent [4]. Extensive use of synthetic insecticides such as: malathion, hexachlorocyclohexane and deltamethrin resulted in disruption of biological control system and severe environmental problems such as: undesirable toxicity signs to non-target organisms [5], development of resistance in mosquitoes [6] and environment pollution [7]. That is why; there is a need for research and development of environmentally safe and biodegradable target specific insecticides. Plants are rich sources of bioactive compounds that are suitable for controlling mosquitoes. Several plant species have been tested as effective and potential when used against different disease vectors [8]. Natural products of plant origin are safer than synthetic insecticides to prevent the diseases caused by mosquito bite [9].

The rice weevil is the major and most destructive pest all over the world in cereal storage. It causes enormous losses in stored cereals and facilitates favorable condition for the growth and appearance of

microorganisms and toxigenic fungi [10]. The use of residual insecticidal protectants (Spinosad, Synthetic pyrethriods, and Organo-phosphorus compounds) is a common preventive measure to protect stored grain from insect damage. However, some grain protectants have high mammalian toxicity and the residues left by these insecticides may cause health concerns because they are conventional neurotoxins that affect human nervous system. In fact, this combined with the development of resistance in many major stored product insect species has resulted in the development and evaluation of residual-risk insecticides, which are more specific to insects and cause less environmental concerns to non-target organisms [11]. Plant derived chemicals are recognized as growth inhibitors or as insecticides, anti-feedants, and repellents. These chemicals, which have no negative effects on the biological environment, are serving as protective agents of the stored grains and bio-degradable products [12-14].

Mallotus repandus (Willd.) Muell.-Arg. (Family-Euphorbiaceae) commonly called Gunti, Jhante or Bon-natai (in Bengali), is a wild species available in Bangladesh and it is used in traditional health practice for treating inflammation, liver-toxicity, ulcer and tumor. The plant also has anti-radical, anti-viral (HIV-1) and uterus muscle stimulant activity [15].

The current investigation is an attempt to evaluate the larvicidal and insecticidal activities of different extracts of *M. repandus* (Willd.) Muell-Arg. Leaf of *M. repandus* has traditional claim for insecticidal activity [16]. To the best of our knowledge no study has been performed to assess this traditional claim of *M. repandus* against mosquito larvae and rice weevil. That is why; we have designed our research project to explore possible larvicidal activity of leaf and stem on mosquito larvae and insecticidal activity on rice weevil.

MATERIALS AND METHODS

Test mosquito and rice weevil: Mosquito larvae of the species *Culex quinquefasciatus* were collected from Insect Rearing and Experimental Station (I.R.E.S.), Department of Zoology, Jahangirnagar University, Savar, Bangladesh. The colony was kept free from exposure to pathogens, insecticides or repellents and maintained at 25–30°C and 75-85% relative humidity for further use. The larvae were fed on 10% sucrose solution and 10% multi-vitamin syrup. Initial stock of *Sitophilus oryzae* was collected from naturally infested rice collected from (I.R.E.S.) laboratory. They were reared under ambient laboratory condition of 28±2ºC and 75±5% relative humidity.

Plant material: The leaves and stems of *Mallotus repandus* (Willd.) Muell.-Arg. were collected from Savar, Dhaka, Bangladesh during the dry season and authenticated by Md. Abdur Rahim, Technical Officer, Department of Botany, Jahangirnagar University. A voucher specimen (DACB Accession No. 38733) was deposited in Bangladesh National Herbarium for future reference.

Preparation of leaf and stem extracts: The collected plant parts of leaf and stem were cleaned and washed well with distilled water. The cleansed leaves and stems were then partially dried by fan aeration and then fully dried in the oven at below 40ºC for 4 days. The fully dried leaves and stems were then grinded to a powdered form and stored in suitable condition for few days. The powdered plant materials of leaf and stem (500 gm) were used for extraction by Soxhlet apparatus at elevated temperature (65ºC) using petroleum ether, ethyl acetate and methanol consecutively (500 ml of each solvent). After each extraction the powder was dried and used again for the next extraction. Extraction was considered to be completed when the plant materials become exhausted of their constituents that were confirmed from cycles of colorless liquid siphoning in the soxhlet apparatus. All three extracts of leaf and stem were filtered individually through fresh cotton bed. The filtrates obtained were dried at temperature of 40±2ºC to have gummy concentrate of the crude extracts. Each extract was kept in suitable container with proper labeling and stored in cold and dry place.

Larvicidal bioassay: The larvicidal bioassay was done following the World Health Organization (WHO) standard protocol with slight modifications [17]. Different concentrations (300, 200, 100, 50, 25 and 5 ppm) of methanol extract of leaf (MLM), ethyl acetate extract of leaf (MLEA), petroleum ether extract of leaf (MLPE), methanol extract of stem (MSM), ethyl acetate extract of stem (MSEA), petroleum ether extract of stem (MSPE) and malathion (0.00018, 0.00037, 0.00075, 0.0015, 0.003, 0.006, 0.012 and 0.024 ppm) were transferred into sterile beaker. Ten of the third instar larvae of *Cx. quinquefasciatus* were separately introduced into different beakers (volume 50 ml) with marked concentrations and the mortality was recorded after 24, 48 and 72 hours of the exposure period. Larvae were considered dead when they failed to move after probing with a dropper in the siphon or cervical region. The experiment was carried out three times and conducted under laboratory conditions at 25–

30°C and 80–90% relative humidity. Each experiment contained one control group. Corrections for mortality were done using Abbot's formula [18]. LC_{50} (Median Lethal Concentration) was calculated using Finney probit analysis [19].

Insecticidal bioassay: Toxicity of the plant extracts on *S. oryzae* was carried out in the laboratory according to the method of Talukder and Howse [20] with some modifications. Five grams of rice were soaked in a 1 ml of 1, 3, 5, 10, 20 and 50% concentrations of six different extracts of *M. repandus* leaf and stem. Concentrations selected for standard deltamethrin were 0.25, 0.5, 1, 3, 5, 10, 20 and 50%. In the control group, rice was treated with solvent only and dried in oven for 30 minutes to evaporate the solvent. Then, each sample was placed in a beaker and ten adults of *S.oryzae* were released on each of the beaker. The experiment was carried out three times. The adult mortality was recorded after 24, 48 and 72 hours of the exposure period. Corrections for mortality were done using Abbot's formula [18]. LC_{50} was calculated using Finney probit analysis [19].

Data analysis: Repeated measures analysis of variance (RM- ANOVA) followed by Tukey multiple comparison was performed to show variation in LC_{50} values of both larvicidal and insecticidal bioassays. LC_{50} values, confidence interval limit, LT_{50} (Median Lethal Time) and chi square values ($\chi 2$) were calculated by Finney probit analysis. The chi square test is used show difference between observed larval mortality and expected larval mortality. Statistical programs which were used were BioStat 2009 (Analyst Soft Inc.) and Graph Pad Prism (version 6.00; Graph Pad Software Inc., San Diego, CA, USA).

RESULTS

Larvicidal bioassay: The results of the present study indicate that MLM, MLPE and MSM showed good LC_{50} values found in three consecutive days (24, 48 and 72 hours). We used malathion as a standard which also showed very good LC_{50} value in the scheduled time. These results of chi square values and 95% lower and upper confidence interval limits are presented in Table 1. We presented an order of sequence according to good LC_{50} values of different extracts and malathion. The order toxicity is Malathion>MLM>MLPE>MSM. Two extracts showed moderate larvicidal potentiality and only MSEA showed poor larvicidal activity. Among six different extracts after 24, 48 and 72 hours of exposure time, MLM showed the highest LC_{50} values of 185.27 ppm having $\chi 2$ of 0.813 in 24 hours and 141.20 ppm having $\chi 2$ of 0.554 in 48 and 72 hours. Moreover, we tested relationship between different times and LC_{50} values of standard and test extracts. Relationship was considered extremely significant ($P<0.0001$). Moreover, LC_{50} values of six different extracts in each specific time were also significantly different ($P<0.05$). We also calculated LT_{50} (50% mortality shown in the fixed time for each specific concentration) and results are shown in Table 2. Among six different extracts MLM, MLPE, MSPE and MSEA exhibited dose dependent lethal time effect. It can be said that values decrease in comparison with the increase in extract concentrations. Low LT_{50} means that the extracts require minimum time to kill larvae. At 300 ppm MLM presented the lowest LT_{50} value in 8.45 hours in comparison with other extracts. Therefore, the extracts are effective at each concentration which requires minimum time to kill larvae.

Insecticidal bioassay: All extracts were effective to kill the insects and showed LC_{50} values in 24, 48 and 72 hours. It was proved that LC_{50} values became low as the time passed. Among all extracts in 24 and 48 hours MSEA showed the highest LC_{50} value of 110.58% having $\chi 2$ of 0.309 in 24 hours and LC_{50} value of 46.35% having $\chi 2$ of 0.968 in 48 hours. On the other hand, MLPE exhibited LC_{50} value of 12.79% having $\chi 2$ of 0.318 in 72 hours. Deltamethrin used as standard showed very good insecticidal activity in the scheduled time. We tested relationship between different times and LC_{50} values of standard and test extracts. Relationship between different times and LC_{50} values were very significant ($P<0.0001$) both for standard and extracts. Moreover, LC_{50} values of six different extracts in each specific time were also significantly different from one another ($P<0.05$). We also calculated LT_{50} (50% mortality shown in the fixed time for each specific concentration). MSM and MSEA showed dose dependent LT_{50}. It was clear to us that values decreased in comparison with the increase in extract concentrations. Lowest LT_{50} value of 52.64 hours was shown by MLPE at 50% concentration among all other extracts. Therefore, the toxicity of the extracts was further substantiated by lethal time effect. All the results are summarized in Table 3 and 4.

DISCUSSION

This study is the first report on larvicidal and insecticidal activities of *M. repandus* stem and leaf. It was found that found that both leaf and stem extracts have larvicidal and insecticidal potentials which substantiated the traditional claim. These

observations may be related to phytochemical constituents present in the leaf and stem extracts. Phytochemicals present in the extracts may show this potential toxicity.

The secondary metabolites present in the plants have wide range of biological activities. Gopieshkhanna and Kannabiran [21] have identified different types of phytochemical constituents in the plant extracts such as: saponins, tannins, flavonoids, etc. having toxicity against mosquito larvae. Saponins work by interacting with the cuticle membrane of the larvae, disarranging the membrane and causing larval death [22]. Isoflavonoid extracted from tubers of *Neorautanenia mitis* showed fruitful activity against *Anopheles gambiae* and *Cx. quinquefasciatus* which are responsible for transmission of malaria and filariasis [23]. Aluminium chloride isolated from alder leaf was reported to have the larvicidal activity against *Ae. aegypti* [24]. Role of tannin compounds as potential larvicidal agent against Cx. *quinquefasciatus* larvae was also reported [21]. Larvicidal activity of alkaloids derived from *Piper longum* fruit and *Triphyophyllum pellatum* was documented by Lee et al. and Francosis et al. respectively [25]. Novel compound β -sitosterol-3-O-β–D-glucoside isolated from *Acanthus montanus* exhibited potent mosquitocidal activity (100% mortality) against adult *Ae. aegypti* [26]. Phrymarolin-I, haedoxane A, and haedoxane E lignans from *Phryma leptostachya* L. showed high larvicidal activity against the early fourth instar larvae of *Cx. Pipienspallens* [27]. Root-derived materials, particularly (−)-asarinin and pellitorine from *Asarum heterotropoides* presented preliminary larvicidal toxicity against *Culex pipienspallens* and *Aedes aegypti* and *Ochlerotatus togoi* and merited further study as potential mosquito larvicides for the control of insecticide-resistant mosquito population [28].

The toxic phytochemicals present in plants kill and inhibit various types of insects [12]. Azadirachtin, isolated from the neem tree (*Azadirachta indica)*, is both toxicant and anti-feedant and one of the most widely tested and successfully implemented plant insecticides [29]. Botanical derivatives oleic acid and linoleic acid showed promising insecticidal activity against fourth instar *Ae. Aegypti* larvae and antifeedent activity against larvae of *Helicoverpa zea*, *Lymantria dispar*, *Orgyia leucostigma* and *Malacosoma disstria* [30]. Extracts of *P. nigrum* were found fruitful with pesticidal activity against rice weevil, *S. oryzae* and cowpea weevil [31]. Estragole, linalool and sabinene extracted from chinese medicinal plant *Zanthoxylum schinifolium* exhibited toxicity activity against *Sitophilus zeamais,* a maize weevil [32]. The essential oil morrilol, 4-vinylguaiacol and acetoanisole, followed by linalool, eugenol and α-caryophyllene obtained from aerial parts of *Amethystea caerulea* L. showed considerable toxicity against maize weevil [33]. Leaf extract of *Datura alba* has scientific basis for the effective application as biorational tool to control stored grain pests such as khapra beetle *Trogoderma granarium* and the rice weevil *Sitophilus oryzae* [34]. Recent studies substantiated the possibility of monoterpenoids as an alternative agent to synthetic insecticides against stored-product pests *Sitophilus oryzae* L. (Coleoptera: Curculionidae), *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrichidae) and *Cryptolestes pusillus* Schönherr (Coleoptera: Cucujidae) [35].

The present findings suggest that the plant extracts have biocontrol potential against mosquito larvae and rice weevil. In larvicidal bioassay, MLM was found to be toxic against *Cx. quinquefasciatus*. In addition, decrease in LT_{50} values in comparison with the increase in concentrations of the same extract further verifies the toxicity of the extract. In insecticidal bioassay, MSEA and MLPE showed the highest insecticidal activity. MSEA presented better LT_{50} than MLPE in each specific concentration. Good LT_{50} indicates that minimum time is needed for each concentration to kill 50% of the larvae which provides strong rationale in support of insecticidal potential of the extracts. Hui and Li reported the isolation of triterpenoid type compounds-lupeol, friedelin, α-amyrin, 3a-hydroxy-13aursan-28,12b-olide, 3b-hydroxy-13a-ursan-28, 12b-olide and its benzoate, D:A-friedo-oleanane lactones and ursolic acid from stem and root bark of *M. repandus* collected from mountainous area of Vietnam [36]. Huang et al. also reported the presence of three new triterpenoids in stem part of *M. repandus* collected from the same area. The author also isolated Bergenin, an isocumamrin from the same plant [37]. Saijo et al. carried out phytochemical study of the leaves of *M .repandus* and isolated four new hydrolyzable tannins, named repandusinin, repandusinic acids A and B, mallotinin together with eight other hydrolyzable tannins and brevifolin carboxylic acid [38]. It would be wise to say that these phytochemicals may be stored in leaf and stem parts of the tested species of *M. repandus* and may contribute to the non selective toxicity against both *Cx. quinquefasciatus* and S. *oryzae*. Therefore, it can be stated that the leaf and stem extracts can work as a nonspecific general toxicants to both insects. Further screening, identification of responsible components for this non specific activity and ascertaining their molecular mechanism of action are necessary to develop an effective vector control agent.

CONCLUSION

In the present study leaf and stem extracts of *M. repandus* showed non-specific toxicity against *Cx. quinquefasciatus* and *S. oryzae*. This finding necessitates development of ecofriendly vector control agent with a few side effects. Moreover, it can be used as an alternative to synthetic insecticides. Isolation of active components from this plant, and the determining of the mode of action of the components are necessary to develop *M. repandus* as effective vector control agent. Furthermore, small scale and semi field trials should be done with a view to evaluating the efficacy of the plant.

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COMPETING INTERESTS

In this research work the authors declare that they have no interest in competing with other researchers.

Table 1. Larvicidal effect of six different extracts of stem and leaf of *M. repandus* against *Cx. quinquefasciatus.* Values are shown as LC_{50} (Mean value) for each extracts and standard in the result columns of 24, 48 and 72 hours.

Extracts/Standard	24 hours	CI	$\chi 2$	48 hours	CI	$\chi 2$	72 hours	CI	$\chi 2$
MSM	502.49[d]	287.97-4944.50	0.177	308.58[c]	208.32-672.41	0.143	296.51[d]	180.99-945.74	0.124
MSEA	948.78[f]	350.36-196809.70	0.367	592.75[e]	271.09-12987.89	0.177	463.07[f]	210.98-17509.37	0.033
MSPE	725.99[e]	304.81-34036.14	2.067	547.86[d]	258.76-9185.36	0.535	310.65[e]	190.36-974.13	0.927
MLM	185.27[b]	124.34-368.24	0.813	141.20[b]	101.74-216.46	0.554	141.20[b]	101.74-216.46	0.554
MLEA	533.45[d]	320.17-126375.76	0.014	780.94[f]	307.71-90014.65	1.639	683.42[g]	279.22-62587.48	0.309
MLPE	374.77[c]	231.63-1146.30	0.635	296.75[c]	177.95-1035.59	0.225	223.36[c]	149.33-473.48	0.024
Malathion	0.004[a]	0.0023-0.0071	8.921	0.0015[a]	0.0004-0.0072	4.387	0.0005[a]	0.0002-0.0007	1.055

Values in same column with different superscripts are significantly different from each other in each specific time ($P<0.05$). RM- ANOVA followed by Tukey multiple comparison was performed to analyze this data set. Overall time effect was considered extremely significant ($P < 0.0001$). Lethal concentration (LC_{50}) was calculated by Finney probit analysis; CI= Confidence Interval limit; $\chi 2$= Chi square

Table 2. Lethal time effect of six different extracts of stem and leaf of *M. repandus* against *Cx. quinquefasciatus*. Values are shown as LT_{50} (Mean).

Extract	5 ppm	25 ppm	50 ppm	100 ppm	200 ppm	300 ppm
MSM	-	156.58	245.27	464.61	82.15	74.95
MSEA	-	20528.47	563.36	398.87	200.87	117.46
MSPE	-	-	1138.82	412.30	239.52	87.36
MLM	-	-	-	-	21.91	8.45
MLEA	-	216.67	123.18	210.46	349.65	162.95
MLPE	-	29952.17	480.97	185.95	89.36	28.93

Standard	0.00018 ppm	0.00037 ppm	0.00075 ppm	0.0015 ppm	0.003 ppm	0.006 ppm	0.012 ppm	0.024 ppm
Malathion	77.624	77.374	65.083	55.784	35.801	22.972	17.924	8.000

Table 3. Insecticidal activity of six different extracts of stem and leaf of *M. repandus* against *S. oryzae*. Values are shown as LC_{50} (Mean).

Extract	24 hours	CI	χ2	48 hours	CI	χ2	72 hours	CI	χ2
MSM	139.78[c]	62.94 - 147733.06	0.096	114.90[c]	57.20-8519.29	0.005	96.14[c]	52.07-1866.50	0.145
MSEA	110.58[b]	38.92-314.13	0.309	46.35[b]	24.32-182.18	0.968	18.31[a]	11.45-37.77	1.276
MSPE	1074.73[f]	1.14-1017525.01	0.002	814.44[d]	6.13-108219.85	0.308	290.49[d]	5.93-14218.57	0.237
MLM	517.16[e]	91.50-44720382438.04	0.286	133.39[c]	52.0-3889.0	0.371	53.50[b]	28.62-259.46	0.692
MLEA	150.90[c]	21.02-1083.10	0.097	106.88[c]	42.91-25094.58	0.066	61.98[b]	29.36-2851.93	0.006
MLPE	229.36[d]	15.18-3465.22	0.006	57.23[b]	26.14-954.08	1.109	12.79[a]	7.48-27.47	0.318
Deltamethrin	12.87[a]	10.31-16.37	1.618	6.02[a]	4.95-7.25	1.170	4.33[a]	1.64-8.89	4.275

Values in same column with different superscripts are significantly different from each other in each specific time (P<0.05). RM- ANOVA followed by Tukey multiple comparison was performed to analyze this data set. Overall time effect was considered extremely significant (P < 0.0001). Lethal concentration (LC_{50}) was calculated by Finney probit analysis.

CI= Confidence Interval limit

χ2= Chi square

Table 4. Lethal time effect of six different extracts of stem and leaf of *M. repandus* against *S. oryzae*. Values are shown as LT_{50} (Mean).

Extract	1%	3%	5%	10%	20%	50%
MSM	-	-	-	-	1702.50	426.12
MSEA	231.30	121.73	86.72	71.54	63.14	56.30
MSPE	-	118.31	123.15	157.12	190.36	126.39
MLM	-	1009.58	572.96	667.49	622.69	70.55
MLEA	-	-	135.69	314.04	240.67	98.33
MLPE	79.40	106.51	82.12	77.71	68.79	52.64

Standard	0.25%	0.5%	1%	3%	5%	10%	20%	50%
Deltamethrin	-	-	85.185	108.589	90.528	23.886	18.626	17.924

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