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Bioactive potentials of *Mansoa alliacea* (Lam.) leaf extracts against three stored-product pests *Callosobruchus chinensis* L.,*Sitophilus oryzae* L. and *Tribolium castaneum* (Hbst.) adults

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ABSTRACT

Petroleum ether (Pet. ether), $CHCl_3$ and CH_3OH extracts of *Mansoa alliacea* (Lam.) leaves were tested against *Callosobruchus chinensis* L., *Sitophilus oryzae* L. and *Tribolium castaneum* (Hbst.) adults through dose-mortality and repellent activity assays. The Pet. ether extract gave LD_{50} values 1.622, 1.015, 0.792, 0.552, 0.483, 0.479, 0.432 and 0.397 mg/cm^2 against *C. chinensis* after 6, 12, 18, 24, 30, 36, 42 and 48 h of exposure; 2.711, 1.799, 1.037, 0.697, 0.596, 0.539 and 0.505 mg/cm^2 against *S. oryzae* after 12, 18, 24, 30, 36, 42 and 48 h of exposure; and 4.275, 1.864, 1.345, 1.10, 0.984, 0.928, 0.866 and 0.799 mg/cm^2 against *T. castaneum* after 6, 12, 18, 24, 30, 36, 42 and 48 h of exposure respectively. The CH_3OH extract gave LD_{50} values 2.349, 1.170, 0.757, 0.644, 0.561 and 0.423 mg/cm^2 against *C. chinensis* after 12, 18, 24, 30, 36 and 42 h of exposure; 5.641, 3.177, 2.706, 2.509, 2.298, 2.116, 2.037 and 1.759 mg/cm^2 after 6, 12, 18, 24, 30, 36, 42 and 48 h of exposure against *S. oryzae* and 1.331, 1.043, 0.863, 0.792, 0.728, 0.636 and 0.612 mg/cm^2 against *T. castaneum* after 12, 18, 24, 30, 36, 42 and 48 h of exposure respectively; while the $CHCl_3$ extract did not show any mortality against all of the three test insects. In the repellency assay, none of the extracts showed any significant repellent activity against any of the test beetles. To show intensity of activity the extracts could be arranged in the following descending order: Pet. ether extract against *C. chinensis*> CH_3OHextract against *C. chinensis*> Pet. ether extract against *S. oryzae* > CH_3OH extract against *T. castaneum*> Pet. etherextract against *T. castaneum*> CH_3OH extract against *S. oryzae.*

1. Introduction

The test plant *Mansoa alliacea* (Lam.), commonly called 'Garlic vine', belongs to the family Bignoniaceae, is popular with its violet coloured flowers and the leaf with pungent garlic-like smell (Ariga and Seki, 2006). This planthas been used traditionally in various pharmaceutical contexts in different countries. In Brazil, the leaf has been used to cure rheumatoid arthritis, dermal infections, and body cleaning purposes (Lanalice and Tavares-Martins, 2016). In Peru, dried leaves of *M. alliacea* are used treat cold, pneumonia and malaria (Desmarchelier *et al*., 1997; Pérez, 2002; Arana, 2005). The resultant liquid of the aerial parts of this plant are also used against fever and in pain due to rheumatic fever (Hasrat *et al*., 1997). Different parts of this plant have been reported for their different medicinal values. Leaves of *M. alliacea* are applied on the skin for analgesic purposes, bark produces as a tincture and the roots are used as a cold maceration (Taylor, 1996). The whole plant extract showed its anti-plasmodial activity at

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500mg/kg body weight (Ruiz *et al*., 2011). Leaves of this plant have been reported for showing antifungal potential against some of the fungal strains for instance- *Alternaria* spp., *Colletotrichum capsica*, *Curvularia lunata*, *Fusarium oxysporum* and *Fusarium udum* (Freixa *et al*., 1998). The root and stem extracts also have been reported for their anti-inflammatory activities (Pires *et al*., 2017). The aqueous extracts of leaf and flower of this plant showed significant larvicidal activity against *Aedes* spp. under laboratory conditions (Rath *et al*., 2013). Leaf extracts of *M. alliacea* exhibited cytotoxic action against cancer cells (Towne*et al*., 2015). One of the test insects *Callosobruchus chinensis* L. commonly known as 'Chinese bruchid, cowpea bruchid and pulse beetle' (Chandra and Girish, 2014). This insect pest generally found in legume industries and causes serious damage to stored pulses (Srinivasan *et al*., 2008). Oviposition of this beetle is influenced by the seed surface smoothness, chemical stimuli and seed coat thickness (Ahmad *et al*., 2018). *Sitophilus oryzae* L., another test insect, occurring throughout the warmer parts of the world and known as 'Rice weevil'. In storage condition, they infest rice, damage the nutritional composition of the grains and produce non-beneficial proteins (Akhter *et al*., 2015). The red flour beetle, *Tribolium castaneum* (Hbst.) is known to be the most destructive pest in granaries, mills, warehouses and stored grains. This insect also considered as the secondary pest of cracked grains. In storage conditions, it decreases the nutritive value of the grains and causes significant economic damages (Burkholder and Faustini, 1991). Since the plant, *M. alliacea* possesses various pharmaceutical, medicinal and biological properties and uses, this investigation was aimed at evaluation of the insecticidal and insect repellent activities of the leaf extracts of this plant against the beetles of the coleopteran pests *C. chinensis*, *S. oryzae* and *T. castaneum*.

2. Materials and method

2.1. Collection and preparation of extracts

Fresh leaves of *M. alliacea* were collected during the month of July 2020 from the University of Rajshahi campus and identified by the herbarium keeper through comparison with the voucher specimen kept in the herbarium of the Department of Botany, University of Rajshahi. The leaves were chopped into small pieces and dried under shade before ground them to powder with the help of an electric grinder. The ground material was weighed and placed in a conical flask to add solvent in a manner of 50g × 150ml × 2 times and the system was then kept on a shaker for 48 h; and filtration was done by using Whatman No. 40 filter paper and the collection was made in the same round bottom flask for the Pet. ether extract. The same was done for the solvents $CHCl_3$ and CH_3OH successively. After evaporation the extracts were removed to glass vials and preserved in a refrigerator at 4ºC with proper labeling.

2.2. Collection of test insects and their culture

Adult beetles of*C. chinensis*, *S. oryzae* and *T. castaneum* were collected from the Crop Protection and Toxicology Laboratory of the Department of Zoology of the University of Rajshahi. A mass culture and sub-cultures of the insects were maintained throughout the experimental period for instant supply of the test beetles

2.3. Bioassays for dose-mortality of *C. chinensis* and *S. oryzae*

The experiments of insecticidal activity assay on *C. chinensis* and *S. oryzae* were the same because of their feeding habit. An *Ad Hoc* experiment was set prior to conduct final experiments to find out the applicable concentrations of the extracts that considered as doses. The doses for the Pet. ether extract against *C. chinensis*were 1.528, 1.273, 1.019, 0.764 and 0.509 mg/cm^2 and for the CH_3OH extract were 1.783, 1.528, 1.273, 1.019 and 0.764 mg/cm^2. The doses for the Pet. ether extract against *S. oryzae* were 1.528, 1. 273, 1.019, 0.764 and 0.509 mg/cm^2 and for the CH_3OH extract were 2.801, 2.546, 2.292 and 2.037 mg/cm^2. One ml of each of the doses was sprayed on the grains and allowed the solvent to dry out. The actual amount of extract present in 1 ml of dose was estimated by dividing the amount with the area of the Petri dish to yield the dose per square centimeter. Being volatile the solvent was rempved quickly. After complete drying the treated food grains kept in the lower part of a Petri dish 10 insects of same age were released in it and covered with the upper lid. The whole system was done in triplicate for replication for each of the doses. A control batch was maintained where the food grains were sprayed with the fresh solvent and evaporated before release of the beetles. The treated Petri dishes were kept in an incubator maintaining the temperature of the stock cultures. The mortality of insects was started counting after 6 h and such collection of data were conducted for more 7 times with 6 h of interval up to 48 h of exposure.

2.4. Bioassay for dose-mortality of *T. castaneum*

- The insecticidal activity assay on *T. castaneum* was not the same as done on *C. chinensis* and *S. oryzae* since the habit of feeding were not the same. An *Ad Hoc* experiment was done to measure out the applicable concentrations considered as doses. The

doses established were 1.528, 1. 273, 1.019, 0.764 and 0.509 mg/cm^2 for the Pet. ether and 1.070, 0.917, 0.764, 0.611 and 0.458 mg/cm^2 for the CH_3OH extracts. One ml of each of the doses was poured down into the lower part of a Petri dish and allowed the solvent to dry out before releasing the insects. Being volatile the solvent was removed, quickly. After complete drying 10 insects of same age were released in it and covered with the upper lid. The whole system was done in triplicate for replication for each of the doses. A control batch was maintained where the lower part on the Petri dish was sprayed with the fresh solvent and evaporated before release of the beetles. The treated Petri dishes with insects were kept in an incubator maintaining the temperature of the stock cultures. The mortality of insects was started counting after 6 h and such collection of data were conducted for more 7 times with 6 h of interval up to 48 h of exposure.

2.5. Statistical analysis

The mortality per cent was corrected by using the Abbott's formula (Abbott, 1925).
P_r = ; Where, P_r = Corrected mortality (%), P_o = Observed mortality (%), P_c = Mortality in the control (%). The data were subjected to Probit analysis (Finney, 1947 and Busvine, 1991).

2.6. Repellent activity

The repellent activity assay was done according to the method of McDonald *et al*. (1970) with some modifications. A certain concentration for each of the extracts of Pet. ether/ $CHCl_3$/ CH_3OH was considered as the stock dose established through *Ad Hoc* experiment to be applied on the food supplied to the beetles of *C. chinensis* or *S. oryzae*, and other successive doses were prepared through serial dilution that gave 0.1571, 0.0785, 0.0392, 0.0196 and 0.0098 mg/cm^2. For the repellent activity of the extracts against *T. castaneum* the doses established separately but through the procedure same as above. For repellency assay on *C. chinensis* and *S. oryzae* the lower part of the Petri dish (9cm diam.) was divided into three parts by placing two narrow sticks stuck on the floor with the help of adhesive tapes, and both the parts on either of the sides were filled with treated and non-treated foods leaving the middle part left free where 10 beetles of same age were released. However, in case of *T. castaneum* half filter paper discs (Whatman No. 40, 9cm diam.) were used by cutting it into two pieces, and one of the two halves was treated with a selected dose and allowed the solvent to dry out by exposing it in the air for 30 minutes. Each treated half-disc was attached lengthwise, edge-to-edge to a control half-disc with the help of adhesive tapes and placed the whole on the lower part of a Petri dish and ten insects were released in the middle of each of the filter paper circles. The whole system was done for each of the doses in triplicate to be considered as replication. A control batch was also maintained in the same manner while the treated half-disc was prepared by applying the fresh solvent and evaporating it immediately. Repellency of beetles was observed for every hour with interval up to five successive hours of exposure. In case of *C. chinensis* and *S. oryzae* data was collected by counting the number of insects from the non-treated part and the middle part of the floor of the Petri dish; while for *T. castaneum* it was done by counting the number of insects found on the non-treated part of the filter paper spread on the floor of the Petri dishes. The data were subjected to calculate percent repulsion, which was again developed by arcsine transformation for the calculation of the ANOVA. The average of the counts was converted to percentage of repellency (PR) using the formula of Talukder and Howse (1993, 1995): PR = (Nc-5) × 20; where, Nc is the average hourly observation of insects on the non-treated halves of the filter paper discs.

3. Results

3.1. Dose mortality ofthe beetles of *C. chinensis, S. oryzae* and *T. castaneum*

The results of dose-mortality assay of Pet. ether, $CHCl_3$ and CH_3OH extracts of *M. allicea* leaves against *C. chinensis*,*S. oryzae* and *T. castaneum* beetles are presented in Table 1.

Table 1. LD_{50} of *M. allicea* leaf extract against *C. chinensis, S. oryzae* and *T. castaneum* adults.

Test insects	Solvent used	LD_{50} (mg cm^{-2}) on different exposures (in hours)							
		6	12	18	24	30	36	42	48
C. chinensis	Pet. ether	1.622	1.015	0.792	0.552	0.483	0.479	0.432	0.397
	$CHCl_3$					-			
	CH_3OH	-	2.349	1.170	0.757	0.644	0.561	0.423	-
S. oryzae	Pet. ether	-	2.711	1.799	1.037	0.697	0.596	0.539	0.505

	$CHCl_3$					-			
	CH_3OH	5.641	3.177	2.706	2.509	2.298	2.116	2.037	1.759
	Pet. ether	4.275	1.864	1.345	1.10	0.984	0.928	0.866	0.799
T. castaneum	$CHCl_3$					-			
	CH_3OH	-	1.331	1.043	0.863	0.792	0.728	0.636	0.612

The LD_{50} values of *M. allicea* leaf extracts (of Pet. ether) against *C. chinensis* were 1.622, 1.015, 0.792, 0.552, 0.483, 0.479, 0.432 and 0.397 mg/cm^2 after 6, 12, 18, 24, 30, 36, 42 and 48 h of exposure were 2.349, 1.170, 0.757, 0.644, 0.561 and 0.423 mg/cm^{2}after 12, 18, 24, 30, 36 and 42h of exposure respectively (of CH_3OH). Against *S. oryzae* the LD_{50} values established for the Pet. ether extract were 2.711, 1.799, 1.037, 0.697, 0.596, 0.539 and 0.505 mg/cm^2 after 12, 18, 24, 30, 36, 42 and 48 h of exposure and for the CH_3OH extract were 5.641, 3.177, 2.706, 2.509, 2.298, 2.116, 2.037 and 1.759 mg/cm^{2}after 6, 12, 18, 24, 30, 36, 42 and 48 h of exposure respectively. Against *T. castaneum* the Pet. ether extract showed LD_{50} values 4.275, 1.864, 1.345, 1.10, 0.984, 0.928, 0.866 and 0.799 mg/cm^2 after 6, 12, 18, 24, 30, 36, 42 and 48 h of exposure and for the CH_3OH extract 1.331, 1.043, 0.863, 0.792, 0.728, 0.636 and 0.612 mg/cm^2 after 12, 18, 24, 30, 36, 42 and 48 h of exposure respectively. 6 and 0.612 mg cm^{-2} after 12 h giving 7 readings of 6 h interval up to 48 h of exposure. However, the $CHCl_3$ extract of *M. allicea* leaf did not show mortality against all of the test insects of *C. chinensis*, *S. oryzae* and *T. castaneum* at all. The intensity of lethality showed by the extracts could be arranged in the descending order of the Pet. ether extract against *C. chinensis*> CH_3OHextract against *C. chinensis*> Pet. ether extract against *S. oryzae* > CH_3OH extract against *T. castaneum*> Pet. etherextract against *T. castaneum*> CH_3OH extract against *S. oryzae.*

3.2. Repellency againstthe adult beetles of *C. chinensis, S. oryzae* and *T. castaneum*

The repellency assay revealed that none of the Pet. ether, $CHCl_3$, and CH_3OH extracts of *M. allicea* leaves were found to possess components repulsive against the adult beetles of the storage pests *C. chinensis,S. oryzae* and *T. castaneum,* and the data are presented in Table 2.

Table 2. ANOVA for repellency by the Pet. ether, $CHCl_3$ and CH_3OH extracts of *M. allicea* leaves against *C. chinensis, S. oryzae* and *T. castaneum* adults

Plant part	Insects used	Solvents used	Source of Variation	Sum of Squares	df	MS	F	P-value
M. allicea (leaves)	*C. chinensis*	Pet. ether	Bd	737.242	4	184.311	4.853 ^{ns}	0.009
			Bti	680.449	4	170.112	4.479 ^{ns}	0.013
		$CHCl_3$	Bd	196.033	4	49.008	1.117 ^{ns}	0.383
			Bti	309.091	4	77.273	1.762 ^{ns}	0.186
		CH_3OH	Bd	380.037	4	95.009	1.143 ^{ns}	0.372
			Bti	232.647	4	58.162	0.700 ^{ns}	0.603
	S. oryzae	Pet. ether	Bd	2287.613	4	571.903	2.701 ^{ns}	0.068
			Bti	5044.28	4	1261.07	5.956 ^{ns}	0.004
		$CHCl_3$	Bd	1765.407	4	441.352	0.920 ^{ns}	0.476
			Bti	4253.47	4	1063.368	2.217 ^{ns}	0.113
		CH_3OH	Bd	3498.967	4	874.742	2.940 ^{ns}	0.053
			Bti	2719.936	4	679.984	2.286 ^{ns}	0.105
	T. castaneum	Pet. ether	Bd	2493.174	4	623.293	1.166 ^{ns}	0.362
			Bti	1745.461	4	436.365	0.816 ^{ns}	0.533
		$CHCl_3$	Bd	8002.453	4	2000.613	2.093 ^{ns}	0.129
			Bti	1985.574	4	496.393	0.520 ^{ns}	0.723
		CH_3OH	Bd	1756.911	4	439.228	1.588 ^{ns}	0.226
			Bti	849.795	4	212.449	0.768 ^{ns}	0.561

Bd= Between doses; Bti= Between time interval; ns = Non-significant.

4. Discussion

After conducting this investigation, we found the potentiality of *M. alliacea* leaf to the stored product pests. Previous Scientists were also conducted many types of research on the efficacy of *M. alliacea* in different aspects that support our findings. Pandya *et al.* (2012) described the antiinflammatory, fungicidal, antibacterial and cholesterol lowering benefits of this plant species. Khurana and Bhargava (1969) revealed that *M. alliacea* extract showed mild biological affectivity against mosaic papaya and ringspot viruses. Towne *et al.* (2015) reported that *M. alliacea* has been used for treating cancer in the town of Puyo in Ecuador. They also described that this plant contains organosulfur ingredients as the same as in garlic, which are correlated with the lower incidence of cancers in clinical trials (Thomson and Ali, 2003; Wang *et al*., 2010). Since the presence of sulfonic compounds both in garlic and *M. alliacea* have some biological properties, the chemical component allicin found in this plant is reported for its antimicrobial activities (Towne *et al.*, 2015). Song and Milner (2001) mentioned that the chemical allicin found in garlic has anti-cancer and antimicrobial properties. Allicin and the derivative allyl sulfide constituents found in *M. alliacea* have been detected with tumour suppression ability (Song *et al*., 2001; Zhou and Mirvish, 2005). Silva *et al.* (2019) described that *M. alliacea* has potentiality against the fungi cause diseases in *Passiflora edulis*. They proved that the alcoholic extract of this plant reduced the growth of the mycelium of the fungi *Fusarium oxysporum* and *Colletotrichum gloeosporioides*. Ameenabee *et al.* (2020) reported that the derivatives of this plant have some pharmacological properties for instance- anti-oxidant, anti-inflammatory, antiseptic, and anti-bacterial. They also revealed that *M. alliacea* used for epilepsy, skin grievance, renal distress, sickening cell disease, helminthic infections, leprosy, tumours etc.

5. Conclusion

The results on the efficacy of the extracts of *M. alliacea* leaves against theinsect pest storage through dose-mortality and repellent activity tests depicted that it contains some compounds with insecticidal potentials, however, there is no potential of being used as repellents. Further studies on the revelation of the responsible compounds in the leaves of this plant are recommended until the end products can be furnished and reached to the common people.

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