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Research Article

Insecticidal Activities of the Leaf Powders of *Chromolaena odorata* and *Mimosa diplotricha* against the Common Bed Bug, *Cimex lectularius* (L.)

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Abstract

Background and Objective: Bed bugs, *C. lecturalius* are small hematophagous parasitic insects that feed on man, bats, chickens and other domestic animals causing severe irritation, inflammation, anaemia and general body discomfort. Studies on the use of invasive alien plants in the control of insect pests are not uncommon. Therefore present study investigated the insecticidal activities of *C. odorata* and *M. diplotricha* leaf powders against the adults of the common bed bug, *C. lectularius*. **Materials and Methods:** Five unsexed adult *C. lectularius* were exposed to different concentrations (0.0, 0.5, 1.0, 1.5 and 2.0 g) of *C. odorata* and *M. diplotricha* leaf powders for 24, 48, 72, 96, 120, 144 and 168 h after which percentage mortality was calculated. **Results:** Mortality of *C. lectularius* caused by the leaf powders of *C. odorata* and *M. diplotricha* was considerably high and observed to be concentration and exposure time dependent. At the highest concentration (2.0 g), *C. odorata* and *M. diplotricha* leaf powders accounted for 72 and 71% mortality of *C. lectularius*, respectively following a 168 h exposure period. **Conclusion:** In sum, this study is the first to demonstrate the insecticidal efficacy of *C. odorata* and *M. diplotricha* leaf powders against the common bed bug, *C. lectularius* and further suggests their usage in the control of the pest in Nigeria and elsewhere.

Key words: *Chromolaena odorata*, Invasive alien plants, *Mimosa diplotricha*, leaf powder, bed bugs, toxicity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cimex lectularius (L.) (Hemiptera: Cimicidae), the common bed bug, is a haematophagous arthropod. It is a blood feeding ectoparasite of humans, chickens, bats and occasionally domesticated animals[1]. *Cimex lectularius* along with *Cimex hemipterus* are the two species, most important to man, other species include *Cimex pipistrelli*, *Cimex pilosellus*, *Cimex adjunctus*, *Cimex columbarius* and *Oeciacus hirundinis*[2]. Feeding behaviour in *C. lectularius* coincides with periods of minimal host activity, when bed bugs leave their refugia to feed[1]. However, they will try to feed any time of day or at night if they are hungry enough and if the opportunity is there and are attracted to their host by both the warmth and also the presence of carbon dioxide in their host breath[3]. A well fed adult *C. lectularius* will live between 99 and 300 days in the laboratory[1]. Bed bugs might live in a home or apartment for at least several months[1]. An adult *C. lectularius* fully engorges in 10-20 min after which it returns to its refugium[1].

Bed bugs reproduce by a gruesome strategy named "traumatic insemination" in which the male stabs the female's abdomen and injects sperm into the wound[1]. During their life cycle, females' can lay more than 200 eggs[4]. The eggs are tiny about the size of a dust particle, whitish and hard to see without magnification, the eggs are sticky, causing them to adhere to surfaces, in about a week; the eggs hatch[1]. *Cimex lectularius* in the home, in the daytime, can be found in cracks in walls, inside seams of luggage, bags and clothes, inside bedding, box springs and furniture[1,4]. They are not evenly distributed but are concentrated in locations called harborages[1].

The medical significance of *C. lectularius* is most commonly attributed to itching and inflammation from their bites which can reduce the quality of life by causing discomfort, sleeplessness, anxiety and embarrassment, thereby affecting emotional health and well-being of certain individuals[3-5]. A large infestation may cause serious harm over time to very young children, to the very old or to the sick and the damage is simply the unacceptable loss of blood[1,4]. Feeding wounds caused by bed bugs may cause secondary infections to enter a host, yet such secondary infections are infrequently reported[2]. Bed bugs infestation results in multi-million dollar damages in the hospitality industry, poultry industry, private and communal households[3]. Some of the economic importance of *C. lectularius* is the costs of pest control, damage to social reputation and replacement of infested infrastructure. The problems in poultry farms include loss of productivity via the allergic reactions by workers, reduced egg value due to bug's fecal spots, lower egg production from affected chickens and increased feed consumption[1].

Previously, *C. lectularius* were common in the developing world but rare in the developed world[5]. The increase in the developed world may have been caused by increased international travel, resistance to insecticides and the use of new pest control methods that are not affective against bed bugs[6]. Because bed bugs hide (doing so in places difficult to access) and do so in the living places of humans, many pesticides and many means of application are ineffective[1]. Fumigation of homes, particularly with pesticides with residual action, is most often recommended but over the years, resistance to many pesticides and their harmfulness to humans have been a cause of concern[6,7].

The use of plant parts such as leaf powders from plant to control insect pests is increasingly being practiced in developing countries possibly because of the unaffordability of conventional insecticides[8]. *Chromolaena odorata* (L.) King and Robinson (Asteraceae) is an invasive alien plant known in Nigeria as Awolowo, Akintola or a Queen Elizabeth weed and it is a perennial weedy shrub native to the Americas from southern Florida to northern Argentina including the Caribbean island[9]. The plant has been documented in several articles as an effective plant in the control of insect pests[10-13]. *Mimosa diplotricha* C. Wright ex Sauvalle (Mimosaceae) is a fast growing annual or perennial shrubby leguminous and invasive vine native to the Americas and thought to have been present in Nigeria for over two decades, although the exact time and mode of introduction is uncertain[14]. A recent study[15] suggested that *M. diplotricha* possesses some insecticidal activities. Studies on the insecticidal activities on the leaf powders of *C. odorata* and *M. diplotricha* against *C. lectularius* are non-existent. Therefore, the aim of this study was to determine the insecticidal activities of the leaf powders of *C. odorata* and *M.* against *C. lectularius*.

MATERIALS AND METHODS

Collection and preparation of plant powders: *Chromolaena odorata* and *Mimosa diplotricha* plants were collected respectively from an open farmland at the Dentistry quarters, within the vicinity of the University of Benin Teaching Hospital (UBTH), Benin City (6°39'N', 5°56'E). Following collection, the leaves were removed from the plants by hand, washed with running water and shade dried for approximately 7 days and thereafter oven dried at 60°C for 72 h. The dried leaves were further blended into a fine powder using an electric blender

(Braun Multiquick Immersion Hand Blender, B White Mixer MR 5550CA, Germany) and then preserved in an air-tight and water-proof container for further use.

Insect collection: Adult bed bugs (*C. lectularius*) used for this experiment were collected from several infested areas such as cracks and crevices in walls, beds, iron and wooden chairs and also iron bunks from several rooms in one of the Halls of residence (Aminu Kano Hall), University of Benin, Benin, Nigeria. Following collection, the insects where morphologically identified by a medical entomologist in the Department of Animal and Environmental Biology after which they were maintained in a 200 mL transparent plastic container stuffed with detached foam (to enable the insects (=bed bugs) aggregate) at an ambient temperature of 25±2°C and RH of 78% in the Laboratory of the Department of Animal and Environmental Biology, University of Benin, Benin. Experiments were conducted 24 h after collections. The trial was conducted in the Laboratory of the Department of Animal and Environmental Biology, University of Benin, Benin, Nigeria, between November, 2016 and March, 2017.

Mortality bioassay: To perform the mortality bioassay, different concentrations (=grams) of the leaf powders of *C. odorata* and *M. diplotricha* (0, 0.5, 1.0, 1.5 and 2.0 g) were weighed and added into a 100 mL transparent plastic container after which the top part of the container was covered with a sheet of paper and tightly sealed with a rubber band. Five unsexed adult *C. lectularius* were introduced into each plastic container through a small hole made on the paper and thereafter, sealed with a paper tape to prevent the insects from escaping. Each concentration including the control was replicated 5 times and arranged in a Completely Randomized Design (CRD) following the methods reported Uyi and Igbinoba[11]. In this way, it was possible to evaluate the efficacy of different concentrations of *C. odorara* and *M. diplotricha* leaf powders against *C. lectularius*. Bed bug mortality for all the treatments (=concentrations) including the control was monitored for a total of 168 h (=7 days). Mortality was recorded after every 24 h and insects were confirmed dead when there was no response to probing with a sharp pin at the abdomen.

Statistical analysis: The effects of exposure durations on mortality levels of *C. lectularius* when treated with four different concentrations (%) of *C. odorata* and *M. diplotricha* root powders were analyzed with General Linear Model Analysis of Variance (GLM ANOVA). When the overall results were significant in the GLM analysis, the difference among the treatments was compared using Tukey's Honest Significant Difference (HSD) test. All data were analyzed using SPSS statistical software, version 16.0 (SPSS, Chicago, USA).

RESULTS

The leaf powders of *M. diplotricha* and *C. odorata* caused some levels of mortality against *C. lectularius*. Mortality levels caused by both plant species ranged between 8 and 72% (Table 1 and 2).

Mortality due to *Mimosa diplotricha* leaf powder: When bugs were treated with the 0.5 g of *M. diplotricha* leaf powder, mortality levels significantly differed ($F_{6,34} = 5.71$; $p = 0.001$) with the 120, 144 and 168 h exposure treatments exhibiting higher mortality levels (40, 48 and 60%, respectively) while the 24 h exposure treatment showed the least mortality level (Table 1). Following the exposure of the bed bugs to 1.0 g of *M. diplotricha* leaf powder at different exposure durations, mortality significantly differed according to exposure duration ($F_{6,34} = 13.96$; $p = 0.001$), with the 120, 144 and 168 h exposure treatments exhibiting higher mortality levels (44, 52 and 64%, respectively) compared to the other exposure treatments (Table 1). Similarly, the mortality of the bugs significantly differed as a function of exposure time, when treated with 1.5 g of *M. diplotricha* leaf powder ($F_{6,34} = 14.17$; $p = 0.001$), with the 120, 144 and 168 h exposure treatments showing higher mortality levels (48, 60 and 67%, respectively) compared to the other exposure treatments (Table 1). Finally, for *M. diplotricha*, the mortality of the bugs differed as a function of exposure duration, when treated with the 2.0 g of the leaf powder ($F_{6,34} = 21.62$; $p = 0.001$), with the 168 h treatment exhibiting the highest mortality (71%) compared to the other exposure treatments (Table 1).

Mortality due to *Chromolaena odorata* leaf powder: When the bugs were treated with the 0.5 g of the leaf powder of *C. odorata* at different exposure durations (24, 48, 72, 96, 120, 144 and 168 h), mortality levels differed on the basis of exposure duration/time ($F_{6,34} = 925.71$; $p = 0.001$), with the 120, 144 and 168 exposure treatments exhibiting higher mortality levels (43, 47 and 60%, respectively) compared to the other exposure treatments (Table 2). Similarly, mortality levels significantly differed according to exposure durations when the bed bugs were treated with the 1.0 g of *C. odorata* leaf powder ($F_{6,34} = 11.14$; $p = 0.001$), with the 120, 144 and 168 h

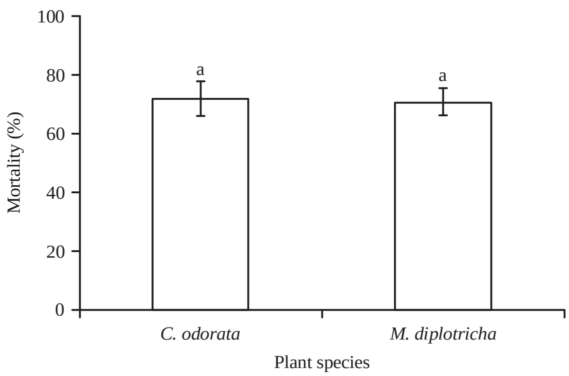

Fig. 1: Mortality (%) (Mean±SE) of *Cimex lectularius* exposed to 2.0 g of *Mimosa diplotricha* leaf powder for 168 h

Means capped with the same letters are not significantly different (student's t-test: p>0.05)

Table 1: Mortality (%) (Mean±SE) caused by different concentrations of the leaf powder of *Mimosa diplotricha* plants against *Cimex lectularius* exposed for 24, 48, 72, 96, 120, 144 and 168 h

Exposure time	Concentration (g) 0.5	1.0	1.5	2.0
Control	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±00*
24 h	8.00±1.89^{a}	12.12±1.40^{a}	16.00±2.50^{a}	20.05±0.00^{a}
48 h	24.50±3.60^{ab}	28.38±3.80^{ab}	28.50±3.88^{ab}	28.00±2.88^{ab}
72 h	27.50±4.80^{ab}	32.50±6.00^{abc}	36.00±4.00^{ab}	40.00±0.00^{bc}
96 h	32.00±3.20^{abc}	36.00±4.80^{bc}	44.50±4.00^{bc}	48.25±4.89^{cd}
120 h	40.00±6.32^{bc}	44.00±4.00^{bcd}	48.00±4.88^{bcd}	52.50±4.88^{cd}
144 h	48.00±4.89^{bc}	52.00±4.88^{cd}	60.00±6.30^{cd}	64.00±4.00^{de}
168 h	60.00±4.52^{c}	64.50±4.00^{d}	67.00±4.89^{d}	71.00±4.89^{e}
F-statistic	$F_{6,34} = 5.71$	$F_{6,34} = 13.96$	$F_{6,34} = 14.17$	$F_{6,34} = 21.62$
p-value	0.001	0.001	0.001	0.001

Means within a column followed by the different letters are significantly different [Tukey's Honest Significant Difference (HSD) test: p>0.05], *Control treatments were not included in the statistical analyses because they showed 0.00% mortality at all exposure durations

Table 2: Mortality (%) (Mean±SE) caused by different concentrations of the leaf powder of *Chromolaena odorata* plants against *Cimex lectularius* exposed for 24, 48, 72, 96, 120, 144 and 168 h

Exposure time	Concentration (g) 0.5	1.0	1.5	2.0
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
24 h	23.00±2.60^{a}	24.50±2.00^{a}	28.00±2.89^{a}	32.00±4.50^{a}
48 h	28.00±4.00^{ab}	28.00±3.80^{ab}	32.00±3.89^{ab}	36.50±4.88^{ab}
72 h	34.00±4.88^{abc}	36.00±4.00^{abc}	40.00±0.00^{abc}	44.50±4.98^{abc}
96 h	39.50±4.88^{abc}	40.00±0.00^{abc}	44.50±4.00^{abc}	48.00±0.00^{abc}
120 h	43.30±4.00^{bc}	44.50±4.00^{bc}	48.50±4.89^{bc}	56.00±5.20^{bcd}
144 h	47.00±5.70^{cd}	48.00±4.88^{cd}	56.00±4.00^{cd}	64.40±4.60^{cd}
168 h	60.50±0.00^{d}	64.00±4.00^{d}	68.00±4.89^{d}	72.00±4.89^{d}
F-statistic	$F_{6,34} = 925.71$	$F_{6,34} = 11.14$	$F_{6,34} = 10.42$	$F_{6,34} = 8.45$
p-value	0.001	0.001	0.001	0.001

Means within a column followed by the different letters are significantly different [Tukey's Honest Significant Difference (HSD) test: p>0.05], *Control treatments were not included in the statistical analyses because they showed 0.00% mortality at all exposure durations

exposure treatments showing higher mortality levels (44, 48 and 64%, respectively) compared to the other exposure treatments (Table 2). When treated with the 1.5 g of *C. odorata* leaf powder at different exposure durations, the mortality of bed bugs significantly differed according to exposure duration ($F_{6,34} = 10.42$; p = 0.001), with the 120, 144, 168 h recording higher mortality levels (48, 56 and 68%, respectively) compared to the other exposure treatments (Table 2). Finally, mortality levels of bed bugs differed as a function of exposure duration when treated with the 2.0 g of *C. odorata* leaf powder ($F_{6,34} = 8.45$; p = 0.001), with the 168 h exposure treatment showing the highest mortality (72%) compared to the other exposure treatments (Table 2).

Comparison of the mortality levels: Mortality did not differed as a function of plant species (Mean±SE, *C. odorata*: 72.51±3.42, *M. diplotricha*: 71.37±3.62, $t_{1,8} = 0.130$; p = 0.896), as both the leaf powders of *M. diplotricha* and *C. odorata* exhibited equal mortality levels (Fig. 1).

DISCUSSION

This study examined the insecticidal activities of *C. odorata* and *M. diplotricha* leaf powders against the adults of the common bed bug, *C. lectularius*. This study was conducted with the aim of finding sustainable alternatives to the use of synthetic insecticides. The results from this study revealed that the leaf powder of *C. odorata* exhibited significant insecticidal activity against *C. lectularius*, although, the insecticidal activity was a function of both concentration and exposure period. Studies focusing on the insecticidal activities of the leaf or root powders and extracts of *C. odorata* against insect pests such as *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae), *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) are not uncommon[10-12] but studies focusing on the insecticidal activities of the leaf powder of *C. odorata* against *C. lectularius* are still scare. The high mortality (72%) of *C. lectularius* caused by the highest concentration (2.0 g) of *C. odorata* leaf powder as observed in this study, corroborates with the findings of other authors[11-16], who reported high mortalities with increasing concentrations of plant powders and extracts against insect pests. As is common with other studies[11,12], *C. lectularius* mortality increased with an increase in the exposure period of the pest to the leaf powder of *C. odorata*. Following a 168 h exposure period, bed bug mortality increased considerably when compared to other exposure intervals, thus corroborating with the findings of Lawal *et al.*[12] who investigated the insecticidal activity of the methanolic leaf extracts of *C. odorata* against the adults of *S. zeamais*.

Empirical evidence on the insecticidal activity of *M. diplotricha* against insect pests have received little[15] or no attention. Therefore, this study is the first to document the insecticidal activity of *M. diplotricha* leaf powder against *C. lectularius.* In accordance with the findings of other studies[10,11], *C. lectularius* mortality after a 168 h exposure period to the highest concentration (2.0 g) of *M. diplotricha* leaf powder was considerably high (71%) and observed to be concentration and exposure time dependent. In addition, *C. lectularius* mortality was observed to have increased with an increase in the duration of exposure of the insect to the leaf powder of *M. diplotricha*. This is in agreement with the findings of other authors[11,17] who documented an increase in insect mortalities with an increase in the exposure time of the pest to the botanicals used.

A number of plausible explanations may account for the mortality of *C. lectularius* caused by the leaf powders of *M. diplotricha* and *C. odorata*. First, plant powders have been reported to control insect pests by eroding the cuticular layer thus leading to dehydration, blocking the spiracles and causing asphyxiation, impairing the respiratory system or by interfering with the metabolism and possibly other systems of the insect's body[18,19]. Second, plants are characterized by the possession of secondary metabolites (=phytochemicals) which have been documented to be toxic to a number of insect pests[20,21]. For example, the leaf extracts of *C. odorata* contain phytochemicals such as alkaloids, saponins, flavonoids and tannins[13,22]. Therefore, it is not impossible to state that the presence of these phytochemicals in the leaves of *C. odorata*, might consequently explain the mortality of *C. lectularius* reported in this study.

Similarly, the mortality of *C. lectularius* caused by the leaf powder of *M. diplotricha* might not be unconnected to the presence of one or more bioactive compounds in the leaves of the plant as has been documented for other plants including *Mimosa* species[20,21,23,24]. For instance, studies on the phytochemical composition of the leaves and roots of a congener, *M. pudica*, revealed that *M. pudica* contains a number of phytochemicals such as alkaloids, flavonoids, tannins and anthroquinones[24]. While it is thought that the presence of these secondary chemicals might be responsible for the mortality of *C. lectularius* in this study, further studies are needed to elucidate or identify the actual secondary chemicals responsible for the mortality of the insect.

CONCLUSION

Although, the leaf powders of both plants exhibited considerable degrees of mortality against *C. lectularius*, further studies should investigate the insecticidal activities of the root powders and the leaf and root extracts of *C. odorata* and *M. diplotricha* against *C. lectularius*. Further, the use of both plants as biological pesticides portends an avenue for the utilization of both invasive alien weeds in Nigeria.

SIGNIFICANCE STATEMENT

This study discovers the insecticidal activities of the leaf powders of two invasive alien plants, *C. odorata* and *M. diplotricha* that can be beneficial for the control and management of bed bugs, *C. lectularius*. This study uncovers the positive attributes of *C. odorata* and *M. diplotricha* that many researchers were unable to explore. Thus a new environment friendly insecticide can be developed from the findings of this study.

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