REPELLENCE AND TOXICOLOGICAL ACTIVITY OF THE ROOT POWDER OF AN INVASIVE ALIEN PLANT, *CHROMOLAENA ODORATA* (L.) (ASTERACEAE) AGAINST *CALLOSOBRUCHUS MACULATUS* (FAB.) (COLEOPTERA: CHRYSOMELIDAE)

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ABSTRACT

Although several studies have demonstrated the repellent and insecticidal activities of the leaf extracts or powder of the invasive alien plant, Chromolaena odorata against stored product pests, studies focusing on the activities of the roots are scarce. The present study investigated the repellent and insecticidal activity of C. odorata root powder against the cowpea weevil, Callosobruchus maculatus. Beetle infested cowpea grains were exposed to two concentrations (2.43 and 3.98 g) of C. odorata root powder for 12, 24, 36, 48 and 72 hours after which percentage repellence and mortality were calculated. The root powder of C. odorata significantly repelled C. maculatus, and the repellent activity was a function of both concentration and exposure time. Following a 48-hour exposure period, 3.98 g of C. odorata root powder exhibited the highest (91%) repellent activity against C. maculatus. Mortality of C. maculatus caused by the root powder of C. odorata plants was high and also observed to be concentration and exposure time dependent. At a low concentration of 2.43 g, C. odorata root powder accounted for 100 % mortality of C. maculatus after a 72-hour exposure period. This study demonstrates the potential of C. odorata root powder in the control of C. maculatus in Nigeria, and portends an avenue for the utilization of an invasive alien weed in Nigeria.

Keywords: Cowpea, *Chromolaena odorata*, Root powder, Toxicity, Mortality, Repellence, *Callosobruchus maculatus*

INTRODUCTION

The use of botanicals such as extracts or powders from plant parts to control insect's pests is increasingly being practiced in developing countries possibly because of the unaffordability of conventional insecticides (Mishra, 2013). The invasive alien plant, *Chromolaena odorata* (L.) King and Robinson (Asteraceae) is one of the numerous plants used by indigenous people to control insect pests in tropical and sub-tropical countries in Africa and Asia (Obico and Ragragio, 2014; Lawal *et al*., 2015; Udebuani *et al*., 2015). *Chromolaena odorata* is native to the Americas from northern Argentina to southern Florida, USA, including the Caribbean Islands (Uyi *et al.,* 2014). The weed is recognized as one of the world worst weeds due to its invasiveness in tropical and subtropical regions where it threatens agriculture, biodiversity conservation and livelihoods (Zachariades *et al*., 2009; Uyi *et al*., 2014). Following the introduction of the weed into Nigeria in the late 1930s and its subsequent spread, the locals discovered its ethnomedicinal and pesticidal importance (Uyi *et al*., 2014). Beyond the usage of *C. odorata* as an insecticide, it is also well known for its repellent activities against various insect pests (Obico and Ragragio, 2014) including insect-pests of stored products (Onunkun, 2013).

Vigna unguiculata is one of the most important food legume crops in the semi-arid tropics covering Asia, Africa, southern Europe, and Central and South America. Most cowpeas are grown on the African continent, particularly in Nigeria and Niger which accounts for over 66 % of world cowpea production (FAO, 2012). Nigeria, the largest producer and consumer, accounts for 61 % of production in Africa and 58 % worldwide (FAO, 2012). Cowpea consumption complements the mainly cereal diets in countries that grow cowpeas as a major food crop (Phillips *et al*., 2003). Cowpeas provide a rich source of proteins and calories, as well as minerals and vitamins. A cowpea seed consist of 25 % protein and is low in anti-nutritional constituents (Rangel *et al*., 2003).

Insects are a major factor in the low yields of cowpea in Africa, and they affect the tissue components and developmental stage of the plant. In severe infestations, insect pressure is responsible for over 50 % loss in yield (Dugje *et al.,* 2009). *Callosobruchus maculatus* is a major pest of cowpeas, green gram and lentils. The ecology and damage caused by *C. maculatus* has been described by CABI (2014). In the early stages of attack the only symptoms are the presence of eggs cemented to the surface of the pulses. As development occurs entirely within the seed, the immature larval and pupal stages are not normally seen. The adults emerge through windows in the grains, leaving round holes that are the main evidence of damage. Infestation may start in the pods before harvest and carry over into storage where substantial losses may occur. The values of dried pulses are strongly influenced by levels of bruchid infestation in local markets, particularly in sub-Saharan Africa (CABI, 2014). Attack on stored grains by *C. maculatus* significantly reduces the quantity and quality of seeds destined for human consumption and sowing purpose (Baidoo *et al*., 2010). Substantial losses (up to 70 %) occur in storage, especially in rural areas of developing countries, where grain legumes are kept in old sacks or mud bins (Dugje *et al*., 2009).

Callosobruchus maculatus have been controlled using fumigation treatments involving the use of phosphine amongst a host of other conventional insecticides. Although these pesticides eliminate insect-pests, they have also been reported to cause a variety of problems to humans, animals and the environment (CABI, 2014), hence the use of botanicals (plant parts) to control insect pests of stored product is increasingly being practiced (Cobbinah *et al*., 1999; Onunkun, 2013). Most studies on the repellent and insecticidal activities of plants often focus on the leaves of such plants including *C. odorata* (Onunkun, 2013; Lawal *et al*., 2015; Udebuani *et al*., 2015). This might be due to the ease of obtaining leaves from plants, however, plant roots are known to be well defended against animals and as such contains higher amount of toxic secondary chemicals such as alkaloids (Macel, 2011). Although studies on the repellent and insecticidal activities of *C. odorata* leaf extracts or powder against stored product pests are not uncommon (Onunkun, 2013; Lawal *et al*., 2015), studies focusing on the activities of the root powder or extracts are scarce. Therefore, the objective of this study is to determine the repellent and insecticidal activity of the root powder of *C. odorata* against the cowpea beetle, *C. maculatus*.

MATERIALS AND METHODS

Collection and Preparation of Plant Powder: Fresh roots of *C. odorata* plants were collected from an open farmland at Dentistry quarters, within the vicinity of the University of Benin Teaching Hospital (UBTH), Benin City (6º39'N, 5º56'E), Nigeria. Following collection, the roots were chopped separately into pieces, washed with running water and shade dried to a constant weight. The dried roots were blended into fine powder using an electric blender (Braum Multiquick Immersion Hand Blender, B White Mixer MR 5550CA, Germany) and then preserved in an air-tight and water-proof container pending use.

Insect Culture: Mass culture of the insect was done on cowpea grains (purchased from Uselu Market, Benin City, Nigeria) at an ambient temperature of 27 ± 2 °C and 80 ± 5 % RH in the Laboratory of the Department of Animal and Environmental Biology, University of Benin, Benin City, Nigeria. Ten pairs of adult beetles (1 – 3 day old) along with the food were placed in five 4 litre aerated plastic containers (with a screw top lid). Containers (with adult weevils) were kept for 7 days in the laboratory for mating and oviposition. The beetles were removed from the containers and the grains containing eggs laid by the weevils were transferred to separate (but similar) containers and allowed to hatch. Only the newly emerged F_2 generation of unsexed adult weevils were used for the trials.

Repellence Test: The experiment was conducted at an ambient temperature of 25 ± 2 °C and 80 ± 5 % RH in the Laboratory of the Department of Animal and Environmental Biology, University of Benin, Benin City, Nigeria. Two different concentrations of the root powder (2.43 and 3.98 g) of *C. odorata* were used. Prior to the repellence and mortality experiments, the cowpea seeds used in this trial were placed in a plastic container and transferred into a freezer and the container was left for 48 hours. The above procedure was done to ensure that the grains were pest-free before using them for the test. Fifty grams of cowpea grains was placed inside a screw top plastic container (100 ml) and treated with 2.43 or 3.98 g of the root powder. The grains and root powders were mixed before being transferred into a perforated 200 ml plastic cup and then the top was covered with aluminum foil and tightly sealed with a rubber band. Ten 1 – 2 day old unsexed adults of *C. maculatus* were introduced into each cup through a hole made in the foil and sealed with a paper tape to prevent insects escaping. The perforated cup was placed inside a completely enclosed and transparent 2 litre plastic bucket to enable an accurate count of the weevils that exit the treated grains. The treatment was replicated ten times for each concentration (grams) and weevils were exposed for 12, 24, 36 and 48 hours. Control treatments, where the grains were not treated with *C. odorata* root powder were also monitored for 12, 24, 36 and 48 hours. The number of insects leaving the treated grains gives a measure of repellence of the root powders.

Mortality Bioassay: To perform the mortality bioassay, 50 g of cowpea grains was placed inside a screw top 100 ml plastic container and one of the two concentrations (2.43 or 3.98 g) of *C. odorata* root powder was added to the grains inside the container. The grains and root powders were mixed before being transferred into a perforated 200 ml plastic cup and then the top was covered with aluminum foil and tightly sealed with a rubber band. Ten 1 – 2 day old unsexed adults of *C. maculatus* were introduced into each cup through a hole made in the foil and sealed with a paper tape to prevent insects escaping. The perforated cup was placed inside a completely enclosed and transparent 2 litre plastic bucket to enable an accurate count of the weevils that leaves the treated grains. The treatment was replicated ten times for each concentration. The numbers of dead weevils were counted at 12, 24, 36, 48 and 72 hours following the commencement of the experiment. Control treatments, where the grains were not treated with *C. odorata* root powder were also monitored for 12, 24, 36, 48 and 72 hours.

Statistical Analysis: The repellent and mortality effect of two concentrations of *C. odorata* root powders on *C. maculatus* was analyzed with General Linear Model Analysis of Variance (GLM ANOVA). The effects of exposure time of the different treatment types on *C. maculatus* was analyzed with Generalized Linear Model (GLZ) assuming a normal distribution with an identity link function. When the overall results were significant in the GLM analysis, the difference among the treatment means were compared using the Bonferroni's test. All data were analysed using SPSS Statistical software, version 16.0 (SPSS, Chicago, USA).

RESULTS

Repellence of *Callosobruchus maculatus:* The root powder of *C. odorata* exhibited some repellence activity against *C. maculatus* compared to the control treatment (Figure 1). Following a 12-hour exposure of *C. maculatus* to different treatment levels of *C. odorata* root powder, percentage repellence significantly differed ($F_{1,29}$ = 16.68; P = 0.0001) among treatments with the control exhibiting the least repellent activity (3 %) against the weevils, while the other treatments (2.43 and 3.98g) exhibited the highest repellence activity (Figure 1a). Similarly, 2.43 and 3.98g of *C. odorata* root powder exhibited a significantly higher ($F_{1,29}$ = 45.75; P = 0.0001) percentage repellence against the weevils following a 24-hour exposure time relative to the control treatment (Figure 1b). Percentage repellence exhibited by the different concentrations against *C. maculatus* significantly differed ($F_{1,29}$ = 139.43; P = 0.0001) following a 36-hour exposure period, with 3.98g of *C. odorata* root powder exhibiting the highest (72 %) repellent activity (Figure 1c).

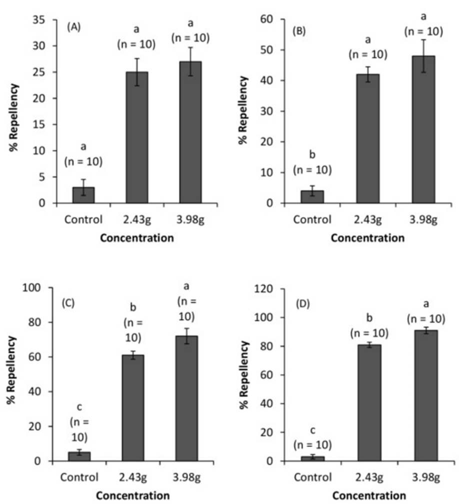

Figure 1: Percentage repellence of *Callosobruchus maculatus* exposed to different concentrations of *Chromolaena odorata* root powder for (A) 12-hours, (B) 24-hours (C) 36-hours and (D) 48-hours exposure periods. Means capped with different letters are significantly different (p<0.05). Sample sizes are given in parenthesis

Similarly, percentage repellence significantly differed ($F_{1,29}$ = 633.09; P = 0.0001) among all three treatments following a 48-hour exposure period, with 3.98g of *C. odorata* root powder exhibiting the highest (91%) repellent activity against *C. maculatus* (Figure 1d). Overall, percentage repellence significantly increased with increased exposure time in the 3.98 and 2.43 g treatments (Table 1, Figure 2).

Table 1: Generalized linear model (GLZ) results for effects of *Chromolaena odorata* root powder, exposure time and their interactions on mortality and repellence of *Callosobruchus maculatus*

Effect	d.f.	Wald $\chi2$	*P*
% Repellence			
Intercept	1	177870.02	0.0001
Treatment	2	73235.31	0.0001
Exposure time	3	27230.14	0.0001
Treatment x exposure time	6	13945.37	0.0001
% mortality			
Intercept	1	183750.24	0.0001
Treatment	2	85696.42	0.0001
Exposure time	4	80486.66	0.0001
Treatment x exposure time	8	39137.33	0.0001

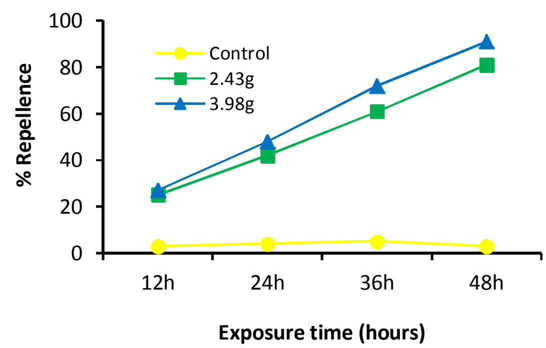

Figure 2: Relationship between percentage repellence of *Callosobruchus maculatus* exposed to different concentrations of *Chromolaena odorata* root powder and varied exposure periods

Mortality of *Callosobruchus maculatus:* Treating *C. maculatus* infested cowpea grains with the root powder of *C. odorata* caused some levels of mortality in the weevils (Figures 3 – 5). When cowpea beetles were exposed for a 12-hour period to different treatments (or concentrations) of *C. odorata* root powder (including control), mortality differed significantly ($F_{2,29}$ = 4.50; P = 0.02) with the control recording no mortality (Figure 3a). Following a 24-hour exposure of the weevils to the different treatments of *C. odorata* root powder, percentage mortality significantly differed ($F_{2,29}$ = 39.09; P = 0.0001) with 2.43 and 3.98 g of the root powder causing higher mortalities (22 and 28 % respectively) relative to the control (Figure 3b). In the 36 hours exposure trial, percentage mortality also significantly differed ($F_{2,29}$ = 195.24; P = 0.0001), with 3.98 g of the powder causing the highest mortality (52 %) followed by the 2.43 g treatment (42 %) and the control (1 %) (Figure 3c). In the 48 hours exposure trial, weevil mortality also differed significantly ($F_{2,29}$ = 504.24; P = 0.0001) among the three treatments with the 3.98g treatment causing the highest (88 %) mortality (Figure 3d).

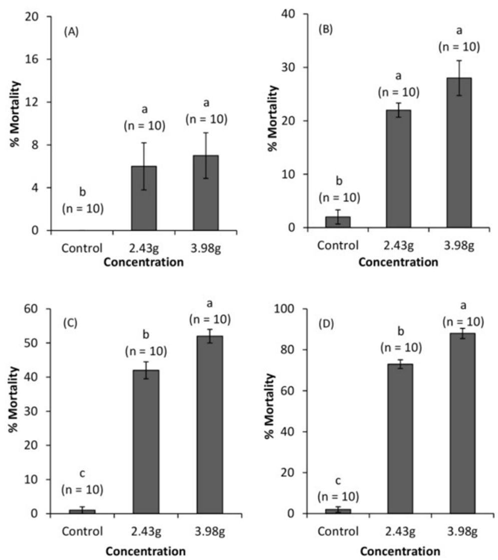

Figure 3: Percentage mortality of *Callosobruchus maculatus* exposed to different concentrations of *Chromolaena odorata* root powder for (A) 12-hours, (B) 24-hours, (C) 36-hours and (D) 48-hours exposure periods. Means capped with different letters are significantly different (p<0.05). Sample sizes are given in parenthesis

Finally, in the 72 hours exposure trial, weevil mortality also significantly differed ($F_{2,29}$ = 5402.25; P = 0.0001) among all three treatments with both 3.98 and 2.43 g *of C. odorata* root powder accounting for 100 % mortality compared to control which only accounted for 2 % mortality (Figure 4). Overall, percentage mortality significantly increased with an increase in exposure time in all treatments except for the control (Figure 5).

DISCUSSION

This study investigated the repellent and insecticidal activity of *C. odorata* root powder on the cowpea beetle, *C. maculatus*. The study was undertaken as part of the wide initiative to find a more environmental and health friendly means of controlling *C. maculatus*, a key pest of *V. unguiculata* in Nigeria and elsewhere. This study revealed that the root powder of *C. odorata* exhibited repellent and insecticidal activities against *C. maculatus*.

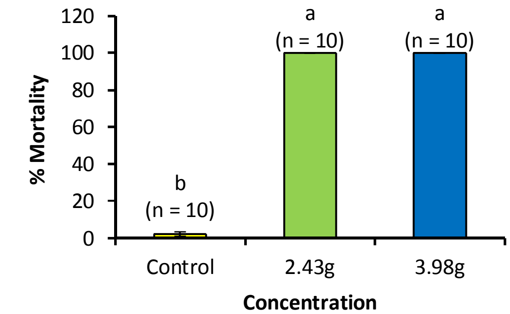

Figure 4: Percentage mortality of *Callosobruchus maculatus exposed to* different treatments of *Chromolaena odorata* root powder for 72-hours exposure period. Means capped with different letters are significantly different (p<0.05). Sample sizes are given in parenthesis

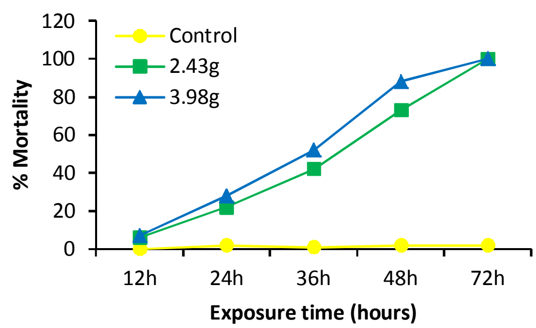

Figure 5: Percentage mortality of *Callosobruchus maculatus* exposed to different concentrations of *Chromolaena odorata* root powder at different exposure periods

This study demonstrated that the root powder of *C. odorata* significantly repelled *C. maculatus*, although the repellent activity was a function of both concentration and exposure time. In Ghana and other West African countries, cowpea grains treated with *C. odorata* leaf powder exhibited repellent activity against adults of *C. maculatus* and *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) (Cobbinah *et al.*, 1999). The high repellent activity demonstrated by the 3.98 g of *C. odorata* root powder supports the findings of other authors (Onunkun, 2013; Udebuani *et al*., 2015; Ahad *et al*., 2016) who reported high repellence with increasing concentrations of plants powders against insect pests including stored product pests. Although several studies have reported the repellent activities of the leaf powder of *C. odorata* against stored product pests (e.g. Onunkun, 2013), but studies on the repellent activities of the root powder of this plant are still scarce. Therefore, this study is the first to report the repellence of the root powder of *C. odorata* against *C. maculatus*. As is common with other studies (Onunkun, 2013; Udebuani *et al*., 2015; Lawal *et al*., 2015), repellence increased with an increase in exposure time.

Although the toxicity of *C. odorata* root powder to *C. maculatus* reported in this study has not been previously documented, but studies reporting the insecticidal activities of the leaf powder and leaf extracts of this plant against stored product pests and other insect pest species are not uncommon (Udebuani *et al*., 2015; Lawal *et al*., 2015). For example, Cobbinah *et al*. (1999) reported that cowpea treated with *C. odorata* leaf powder were free of insect infestation for four months probably because the powder exhibited insecticidal or repellent activities. The high mortality exhibited by the highest concentration of *C. odorata* root powder supports the findings of other authors (Abugri, 2011; Brisibe *et al*., 2011; Rajmohan and Logankumar, 2011; Sukhthankar *et al*., 2014; Lawal *et al*., 2015; Ahad *et al*., 2016) who reported high mortalities with increasing concentrations of either plant extracts or powders against insect pests including those of stored products. As is common with other studies (Sukhthankar *et al*., 2014; Lawal *et al*., 2015; Ahad *et al*., 2016), insect mortality increased with an increase in exposure time. For example, a study conducted by Lawal *et al*. (2015) showed that methanol extracts of *C. odorata* leaf extracts had remarkable insecticidal activity against adult *S. zeamais* after a 96 hour exposure than at 72 hours.

A number of plausible explanations may account for the high repellence and mortality exhibited by *C. odorata* root powder against *C. maculatus* in this study. First, plant powders are known to control insects by eroding the cuticle layer and causing dehydration (Kedia *et al*., 2013); blocking the spiracles and causing asphyxiation (Denloye, 2010) or impairing physiological processes by penetrating the insect body via the respiratory or alimentary system (Ofuya and Dawodu, 2002). The above will ultimately result in high insect mortalities in controlled environment or systems such as silos or other storage structures. Second, plant powders are known to be a rich source of phytochemicals which produce odours that repel adult beetles including *Callosobruchus* species (Ahad *et al*., 2016). The activity of crude plant powders may be as a result of complex mixtures of active chemicals they contained (Sukhthankar *et al*., 2014). Therefore, the presence of these phytochemicals might alter biochemical functions in insects (Udebuani *et al*., 2015). Phytochemicals such as alkaloids, phenols, flavonoids, saponins, cardenolides, anthraquinones and tannins are known to be found in *C. odorata* roots (Agaba and Fawole, 2014; Agaba *et al*., 2015) and these might consequently explain the high mortality of *C. maculatus* reported in our study.

Conclusion: This study demonstrated the potential for the use of *C. odorata* root powder in the control of the cowpea beetle (*C. maculatus*) for the first time. The result of this study further suggests that at a low concentration of 2.43 g, *C. odorata* would reduce infestation of *C. maculatus* at an exposure time of 72 hours. This would be of immense benefit to the farmer as this is a cheaper and more effective means of controlling *C. maculatus* when compared to the use of conventional pesticides. Both *C. odorata* and *C. maculatus* are a threat to agriculture and the use of the former in controlling the latter cancels out the threat posed by the invasive alien plant, thereby resulting in a win-win situation for farmers and agricultural productivity. Although *C. odorata* root powder exhibited high repellent and insecticidal activities, the toxicology behind its potency is poorly understood, hence studies on the phytochemistry of *C. odorata* roots are needed to not only improve our understanding of insecticide science but also to advance our knowledge of controlling stored product pests such as *C. maculatus* in order to reduce or ameliorate the damage caused by this weevil to stored cowpea grains.

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