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Evaluation of the Repellent and Insecticidal Activities of the Leaf, Stem and Root Powders of Siam weed (*Chromolaena odorata*) against the Cowpea Beetle, *Callosobruchus maculatus*

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ABSTRACT: This study investigated the repellency and toxicological activity of *C. odorata* root, stem and leaf powders against adults of the cowpea beetle, *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae). Adults of *C. maculatus* were exposed to grains treated separately with the root, stem and leaf powders of *C. odorata* at different exposure periods of 12, 24, 36, and 48 hours. All the three plant parts significantly repelled *C. maculatus* with the root powder showing the highest percentage repellency, although this was a function of exposure time. The order of repellency after 48 hours exposure was 88, 83 and 76% for the root, leaf and stem powders, respectively. Powders from the three plant parts exhibited insecticidal activity by causing varying levels of mortality to *C. maculatus* with mortality increasing with increase in exposure time. The root powder accounted for the highest adult mortality (74%) while the leaf powder accounted for the least mortality (51%) after 48 hours exposure time. The high repellent and insecticidal activities demonstrated by the root powder compared to the leaf and stem powders suggest that the root powder should be prioritized for the control and management of *C. maculatus.* ©JASEM
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Cowpea, *Vigna unguiculata* (L.) (Walp) (Fabaceae) has sustained millions of peoples in the tropical regions of Africa, Asia and America. It is an extremely valuable crop both as a source of revenue and a cheap source of dietary protein especially in developing countries where meat and fish are expensive (Maina and Lale, 2004; IITA, 2016). It is a complementary to staple cereal and starchy tuber crops (Maina and Lale, 2004; IITA, 2016). Nigeria is the largest producer and consumer of cowpea, accounting for about 58% of the world's production (Maina and Lale, 2004) and 61% of the production in Africa (IITA, 2016). However, production is generally low as a result of serious insect pest attacks which cause heavy losses mostly during storage of the dried grains (Abba, 2013).

One of the most important pests of cowpea is the cowpea bruchid, *Callosobruchus maculatus* (Fab.) (Coleoptera, Chrysomelidae). It causes substantial quantitative and qualitative losses manifested by seed perforation, reduction in weight and market value which renders the grains unfit for human consumption and for sowing purposes (Maina and Lale, 2004; Umeozor, 2005; Bhalla *et al*., 2008). While the control of this pest has relied on chemical methods involving the use of pesticides to treat infested grains (e.g. Agaba *et al*., 2015), these methods have proved ineffective due to resistance issues, persistence of toxic chemical residue on the grains and adverse environmental impacts (Osekre and Ayertey, 2002; Bhalla *et al*., 2008), hence the need to investigate ecologically safe methods to control insect pests of cowpea (Bhalla *et al*., 2008).

In recent years, scientists and locals have increasingly recognized the potentials of a variety of botanicals (extracts and powders from plant parts) to control insect pests (Rajmohan and Logankumar, 2011) including stored product pests (Onunkun, 2013; Lawal *et al*., 2015). One of the plants used by locals to control cowpea weevils is *Chromolaena odorata* (L.) King and Robinson (Asteraceae) (Cobbinah *et al*., 1999), an invasive alien weed that is widespread in Nigeria and other tropical and sub-tropical regions of the world (reviewed in Uyi *et al*., 2014). Although the leaves of *C. odorata* is known to possess some repellent and pesticidal activities (Lawal *et al*., 2015; Udebuani *et al*., 2015), it is unclear whether other parts (e.g. stems and roots) of the plant can equally repel or cause high mortality to cowpea beetles. Therefore the objective of this study was to evaluate the repellent and insecticidal activities of the leaf, stem and root powders of *C. odorata* against *C. maculatus*.

MATERIALS AND METHODS

Collection and preparation of plant powder: Fresh leaves, stem and 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 stem, leaves and roots were chopped separately into pieces, washed with running water and shade dried for about 7 days thereafter oven dried at 60°C for 24 hours. The dried plant was blended into a fine powder using an electric blender (Braun Multiquick Immersion Hand Blender, B White Mixer MR 5550 CA, Germany) and then preserved in an air-tight and water-proofed container for further use.

Insect culture: Mass culture of the insect was reared on cowpea grains (purchased from Uselu Market, Benin City, Nigeria) at an ambient temperature of 26 ± 2 °C and 80 ± 5 % Relative Humidity (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 grains were placed in five 4 litre aerated plastic containers (with a screw top lid). Containers (with adult beetles) 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 beetles 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**.**

Repellency 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. Three different treatment types viz. leaf, stem and root powders were used to evaluate the repellency of *C. odorata* plant against *C. maculatus*. To perform the repellency bioassay, 50g of cowpea grains was placed inside a screw top 100 ml plastic container and powder from a specific plant parts of *C. odorata* (leaf: 0.85g; stem: 1.96g; root: 2.43g) was added to the grains inside the container. The grains and powders were mixed before being transferred into a perforated 200 ml plastic cup. The top of the cup was then 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 beetles that exit the treated grains. The treatment was replicated ten times for each treatment type and the beetles were exposed for 12, 24, 36 and 48 hours. Control treatments, where the grains were not treated with *C. odorata* powders were also monitored for 12, 24, 36 and 48 hours. The number of insects leaving the treated grains gives a measure of repellency of the different powders**.**

*Mortality bioassay***:** To perform the mortality bioassay, 50g of cowpea grains was placed inside a screw top plastic container (100 ml) and powder from a specific plant parts of *C. odorata* (leaf: 0.85g; stem: 1.96g; root: 2.43g) was added to the grains inside the container. The grains and powders were mixed before being transferred into a perforated 200 ml plastic cup. The top of the cup was then 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 the insects from escaping. The perforated cup was placed inside a completely enclosed and transparent 2 litre plastic bucket. Ten replicates were used for each treatment. The numbers of dead beetles were counted at 12, 24, 36, and 48 hours following the commencement of the experiment. Control treatments, where the grains were not treated with *C. odorata* powders were also monitored for 12, 24, 36 and 48 hours.

Statistical analysis: The control treatments, where the grains were not treated with *C. odorata* powder showed 0.0% repellency and mortality of beetles, hence the controls were not included in the statistical analyses. The repellent and mortality effects of the different *C. odorata* powders on *C. maculatus* were 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 treatments was compared using the Bonferroni's test. All data were analysed using SPSS Statistical software, version 16.0 (SPSS, Chicago, USA).

RESULTS AND DISCUSSION

Powders from all three treatment types (leaf, stem and root of *C. odorata* plants) exhibited repellent activity against *C. maculatus* (Table 1; Figure 1). Following a 12-hour exposure period of *C. maculatus* to powders from all three plant parts, percentage repellency did not significantly differ ($F_{2,29} = 3.11$; $P = 0.061$) (Figure 1a). In the 24 hours exposure trial, treatment types had significant effects on the percentage repellency of the beetle ($F_{2,29} = 5.56$; $P = 0.009$) with the root powder exhibiting a significantly higher percentage repellency (44%) against the weevils compared to the stem and leaf powders that exhibited 31 and 28% respectively (Figure 1b). Following a 36-hour exposure of the beetles to powders from the different parts of the plants, the leaf and root powders exhibited a significantly ($F_{2,29} = 4.62$; $P = 0.019$) higher repellent activity (75 and 70% respectively) compared to the stem powder (61%) (Figure1c). Similarly, in the 48-hour exposure trial, the root and leaf powders of *C. odorata* exhibited a significantly ($F_{2,29} = 4.07$; $P = 0.029$) higher percentage repellent activity (88 and 83%

respectively) against the beetles compared to the stem powder that exhibited 76% repellency (Figure 1d). Overall, the repellency of the powders from the three plant parts against *C. maculatus* significantly increased with increased exposure time (Table 1; Figure 2)

Table 1: Generalized linear model (GLZ) results for effects of powders from three different parts (leaf, stem and root) of *Chromolaena odorata* plant, exposure time and their interactions on mortality of, and repellency against *Callosobruchus maculatus*. Following arcsine square root transformation of the data, normal distributions with an identity link function were assumed.

Effect	d.f.	Wald $\chi 2$	*P*
% Repellency			
Intercept	1	334963.33	**0.0001**
Treatment type	2	1946.66	**0.0001**
Exposure time	3	65056.67	**0.0001**
Treatment type x exposure time	6	2653.33	**0.0001**
% Mortality			
Intercept	1	188440.83	**0.0001**
Treatment type	2	4831.68	**0.0001**
Exposure time	3	51775.83	**0.0001**
Treatment type x exposure time	6	2061.65	**0.0001**

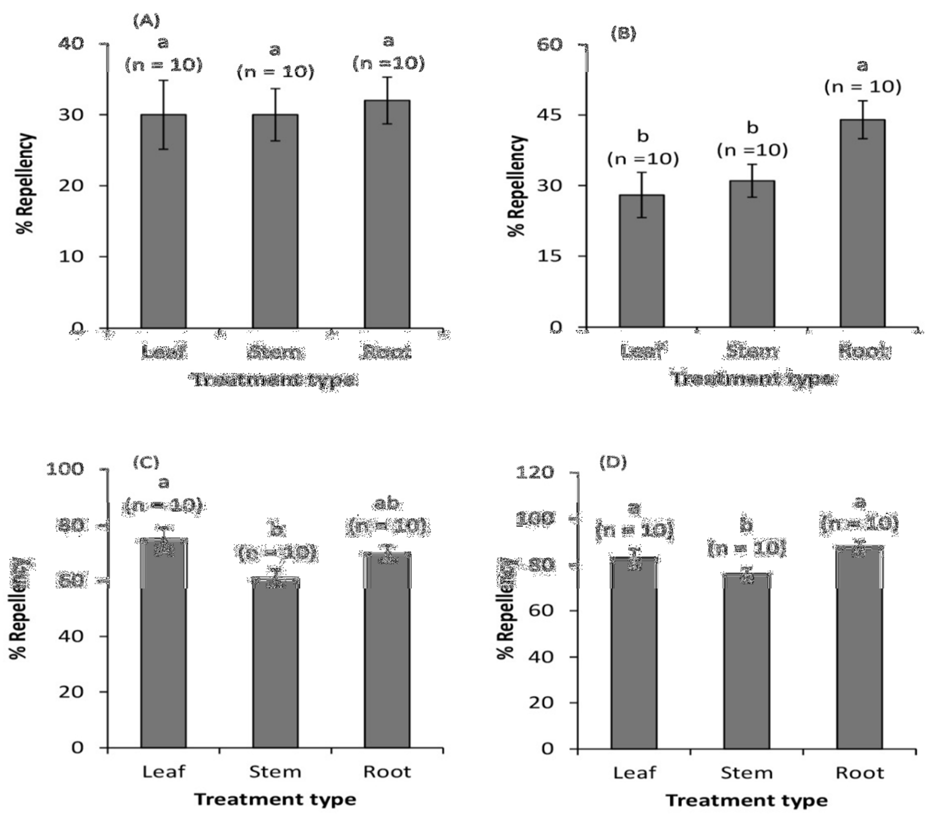

Fig 1. Percentage (mean ± se) repellency of powders obtained from different parts (leaves stems and roots) of *Chromolaena odorata* plants against *Callosobruchus maculatus* exposed for 12 hours (a), 24 hours (b), 36 hours (c) and 48 hours (d). Means capped with the same letters are not significantly different (after Bonferroni's test: P>0.05). Sample sizes are given in parenthesis.

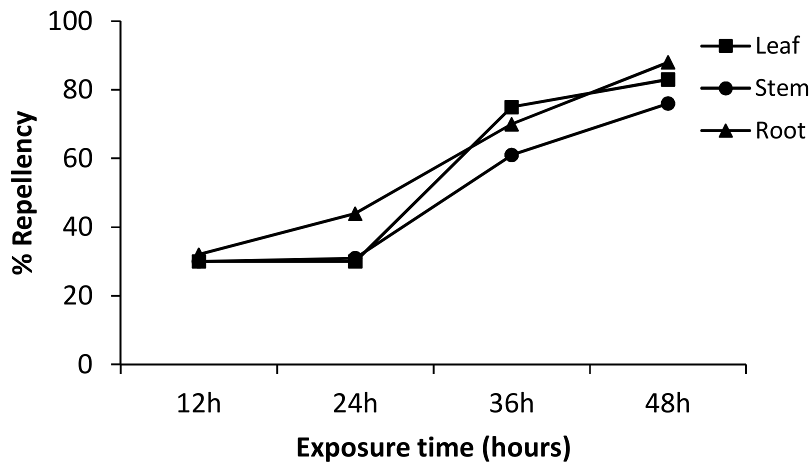

Fig 2: Percentage (mean) repellency of powders obtained from different parts of *Chromolaena odorata* plants against *Callosobruchus maculatus* at different exposure periods (time: 12h, 24h, 36, and 48 h).

This study documented differences in the repellent activities of all three plant parts, with the root and leaf powders showing the highest repellency against *C. maculatus*. Several studies (e.g. Pascual-Villalobos and Robledo, 1998; Onunkun, 2013) have consistently reported the repellent activities of plants belonging to the Asteraceae family. For instance, Sahayraj and Paulraj (2000) observed that *Spodoptera litura* (Fab.) (Lepidoptera: Nuctuidae) larvae were repelled from leaves of groundnut plants treated with *Tridax procumbens* (Linn.) (Asteraceae) leaf extract and the repellency increased with a corresponding increase in the concentration of the leaf extract. Although studies reporting the repellency of *C. odorata* leaf powder against insect pests (including those of stored products) are not uncommon (e.g. Cobbinah *et al*., 1999; Onunkun, 2013), reports on the repellent activities of the stem and root powders of *C. odorata* are still scarce (but see Uyi and Igbinoba, 2016).

In accordance with the findings of Onunkun (2013), there was an appreciable increase in repellency of the leaf powder with an increase in exposure time. Similarly, the repellent activities of the root and stem powders also increased with increase in exposure time. The variability in the repellent activities of the different powders suggest dissimilarities in the concentrations of secondary chemicals in the different plant parts – as the roots are known to possess additional or higher concentrations of secondary chemicals (=constitutive defence) such as pyrollizidine alkaloids (PAs) (Biller *et al*., 1994).

Powders from all three treatment types (leaf, stem and root of *C. odorata* plants) exhibited insecticidal activities by causing varying levels of mortality to *C. maculatus* (Table 1; Figure 3). Following a 12-hour exposure of the cowpea beetles to powders from all the three plant parts, percentage mortality did not differ ($F_{2,29}$ = 0.12; P = 0.888) among the treatments and mortality was below 10% in all treatments (Figure 3a). In the 24 hours exposure trial, the root powder caused significantly ($F_{2,29}$ = 4.09; P = 0.028) higher mortality (27%) to *C. maculatus* compared to the stem and leaf powders which accounted for 17 and 14% respectively (Figure 3b). Mortality of *C. maculatus* significantly differed ($F_{2,29}$=9.38; P = 0.0001) among all treatment types in the 36 hours trial, with the root powder accounting for highest mortality (49%) while the leaf powder recorded the least mortality (24%) (Figure 3c). Finally, mortality also significantly differed ($F_{2,29}$ = 9.11; P = 0.0001) among the treatment types in the 48 hours trial, with the root powder accounting for the highest mortality (74%) while the leaf powder accounted for the least mortality (51%) (Figure 3d). Overall, mortality significantly increased with an increase in exposure time irrespective of treatment types (Table 1; Figure 4).

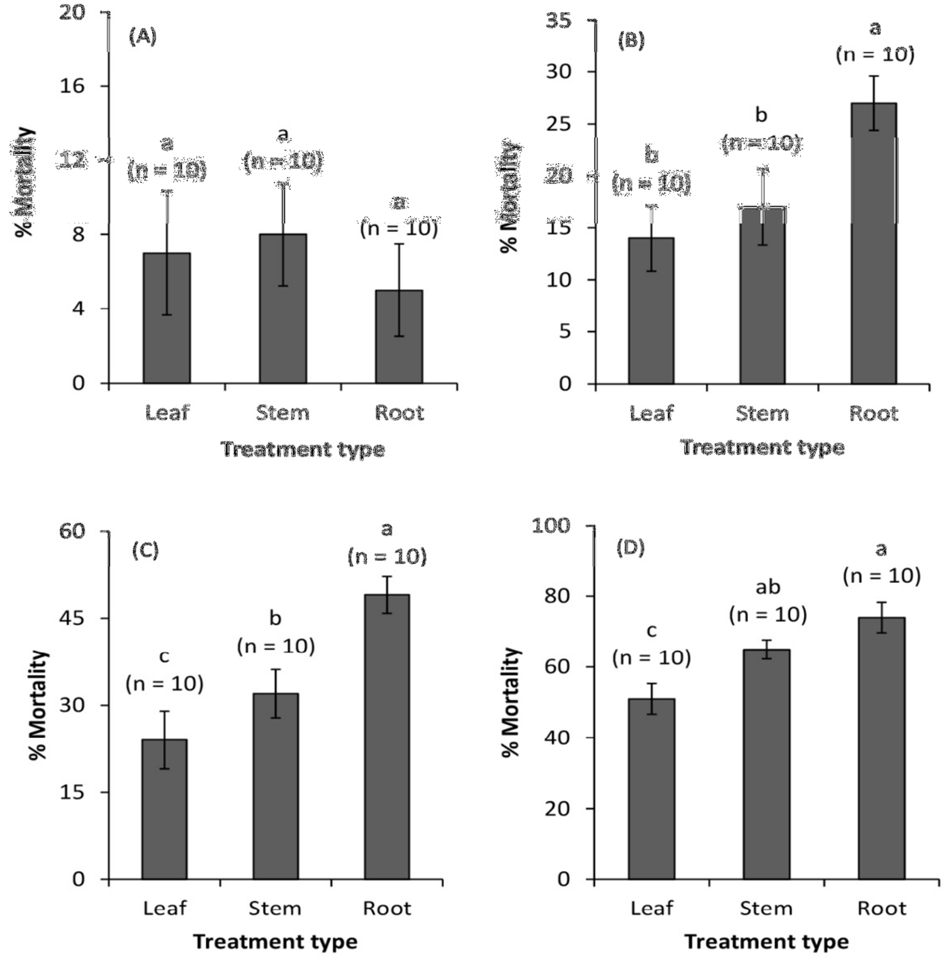

Fig 3: Percentage mortality (mean ± se) caused by powders obtained from different parts (leaves, stems and roots) of *Chromolaena odorata* plants against *Callosobruchus maculatus* exposed for 12 hours (a), 24 hours (b), 36 hours (c) and 48 hours (d). Means capped with the same letters are not significantly different (after Bonferroni's test: P>0.05). Sample sizes are given in parenthesis.

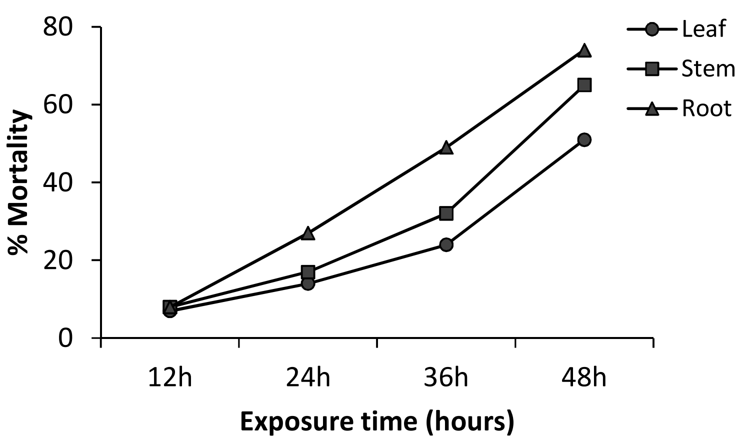

Fig 4: Percentage mortality (mean) caused by powders obtained from different parts of *Chromolaena odorata* plants against *Callosobruchus maculatus* at different exposure periods (time: 12h, 24h, 36, and 48 h).

Cowpea seeds treated with *C. odorata* root powder (compared with other plant parts) resulted in the highest mortality of *C. maculatus*, while the leaf powder recorded the least mortality. Although several studies have reported the insecticidal activities of the leaf extract of *C. odorata* plants against insect pest species (e.g. Rajmohan and Logankumar, 2011; Sukhthankar *et al*., 2014; Udebuani *et al*., 2015), reports on the insecticidal activities of the leaf powder against stored product pests such as *C. maculatus* are not uncommon (Cobbinah *et al*., 1999; Onunkun, 2013). Our study demonstrates that the root and stem powders seemed to be more toxic and significantly caused higher mortalities irrespective of exposure time. However, studies on the efficacy of the root and stem powders against stored product pests are still relatively scarce (but see Uyi and Igbinoba, 2016). The reason why the root powder of *C. odorata* exhibited higher repellent and insecticidal activities against *C. maculatus* is probably due to the presence of higher concentrations of phytochemicals in them (compared to the stems or leaves) as has been documented in an earlier study (e.g. Biller *et al*., 1994). As is common with other reports (e.g. Ahad *et al*., 2016), mortalities in the various treatments increased with an increase in exposure time.

The fact that the different parts of the *C. odorata* exhibited some levels of repellency and insecticidal activities against *C. maculatus* suggests that the plant possesses toxic phytochemicals (secondary chemicals) such as saponins, alkaloids, phenolics, flavonoids, tannins and (Biller *et al*., 1994; also see review in Omokhua *et al*., 2016). Saponins are known to have clear insecticidal properties (DeGeyter, 2012) and causes increased mortality levels, decreased reproduction, reduced level of food intake and weight reduction in insects (De Geyter, 2012). These could be attributed to saponins making foods less attractive to eat (repellent/deterrent activity); causing digestive problems, causing moulting defects or having toxic effects on cells (De Geyter, 2012). Saponin interacts with cholesterol thereby disturbing ecdysteroid synthesis; it also inhibits protease and is toxic to insect cells (Chaieb, 2010). Similarly, phenolic compounds have intrinsic protective abilities against invading organisms; as signal and plant defense molecules (Joachim *et al*., 2007). Alkaloids are complex compounds that occur naturally in plants and are toxic to insects (Fatoki and Fawole, 2000). The alkaloids present in *C. odorata* have shown nematostatic and nematicidal effects on plant-parasitic nematodes (Thoden *et al*., 2009; Agaba and Fawole, 2014). A class of metabolites, the 1,2-dehydropyrrolizidine alkaloids are well-known feeding deterrents against herbivores and are toxic to a wide range of non-adapted animals (Narberhaus *et al*., 2005; Thoden *et al*., 2009); and may have potential for insect pest management. Flavonoids are a class of phenolic compounds that have anti-feeding and attracting deterrent properties, thus are toxic to insects, fungi, nematodes and weeds (Carlsen and Fomsgaard, 2008). Tannins are polyphenols that are toxic to small mammals (Fatoki and Fawole, 2000). Tannins act as a defense mechanism in plants against pathogens and herbivores (Kumbasli *et al*., 2011).

Conclusion: This study demonstrated that all three plant parts of *C. odorata* are significantly toxic to the cowpea beetle, *C. maculatus* at the various exposure times tested. The high repellent and insecticidal activities demonstrated by the root powder (compared to the leaf and stem powder) suggests that the root powder will be more effective in the control and management of the cowpea beetle, *C. maculatus*.

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