INSECTICIDAL AND ANTI-OVIPOSITIONAL ACTIVITIES OF THE LEAF POWDER OF *JATROPHA CURCAS* (L.) (EUPHORBIACEAE) AGAINST *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: CHRYSOMELIDAE)

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ABSTRACT

This study investigated the insecticidal and anti-ovipositional activities of the leaf powder of J. curcas against the cowpea beetle, Callosobruchus maculatus. Five pairs of sexed freshly emerged adults of C. maculatus were exposed to cowpea grains (20 g) treated with different concentrations (0.0, 1.0, 2.0 and 3.0 g) of the leaf powder of J. curcas for 24, 48, 72, 96 and 120 hours after which percentage mortality was calculated. In the same experimental setup, anti-ovipositional activity of the leaf powder of J. curcas was determined by counting the number of eggs on each cowpea grain after 120 hours of mating and oviposition by C. maculatus. Mortality of C. maculatus caused by the leaf powder of J. curcas was high and observed to be independent of the concentrations used, but dependent on the duration of exposure. At all concentrations (excluding the control – 0.0 g), the leaf powder of J. curcas demonstrated significant insecticidal activities (94 – 98 %) against C. maculatus following a 120 hour exposure period. At the lowest concentration (1.0 g), the leaf powder of J. curcas reduced oviposition rate in C. maculatus by 81.04 % after 120 hours of mating and oviposition. In summary, this study suggests the use of the leaf powder of J. curcas as an attractive alternative to conventional insecticides in the control of C. maculatus infestation in Nigeria and elsewhere.

Keywords: Cowpea, *Jatropha curcas*, *Callosobruchus maculatus*, Leaf powder, Mortality, Anti-oviposition

INTRODUCTION

Environmental and ecological problems caused by the persistent use of synthetic insecticides in insect pest control have spurred the search for ecofriendly alternatives (Ghosh *et al*., 2007; Rozman *et al*., 2007). While the use of broad spectrum synthetic insecticides in curbing the menace caused by insect pests have produced fascinating results over the years (Mgbemena *et al*., 2016), recent reports (Musa and Olaniran, 2015; Mgbemena *et al*., 2016), showed that their persistent and uncontrolled usage have led to various unforeseen and undesirable outcomes such as the evolution of resistant strains of insect pests, high cost, unrestricted availability, environmental persistence, hazards to humans and non-target organisms amongst others. Following the public concern on the use and safety of synthetic insecticides in insect pest management, recent pest control measures have been centered on the use of non-chemical techniques including the use of natural physical and biological products. Plant products viz. powders, extracts and oils, offer a vast and virtually untapped or under-tapped reservoir of bioactive compounds with many potential uses (Thoden *et al*., 2009; Chaieb, 2010). These plant bioactive products do not contribute to pest resurgence nor do they enhance the development of resistance in insect pests.

Cowpea, *Vigna unguiculata* is one of the most important food and cash crop legume grown in the tropics (Musa and Olaniran, 2015). It is used to combat malnutrition amongst the rural populace (Ileke *et al*., 2012), where it serves as an inexpensive alternative source of protein to meat, fish and eggs. Cowpea is also a complementary diet to cereal consumption (Phillips *et al*., 2003). The annual production of cowpea in the World is estimated at 4.5 million metric tons (Musa and Olaniran, 2015). In spite of this, its continuous production all over the world have been under severe threats by the activities of several insect pests which cause considerable losses both in the field and in storage (Uyi and Igbinoba, 2016).

The cowpea beetle, *Callosobruchus maculatus* is an ill-famed pest of cowpea and other leguminous grains (Rahman and Talukder, 2006). The beetle causes substantial qualitative and quantitative pre- and post-harvest losses in varying degrees in the tropics (Gbaye *et al*., 2011). These losses are usually manifested by the egg laying and feeding behaviors of the adult females and larval stages of the insect respectively. When gravid, female *C. maculatus* lays its eggs on ripening cowpea pods and seeds, which is then followed by the emergence of the larvae under favorable conditions. The emerging larvae burrow through the chorion of the eggs into the seeds, thereafter, feeding on the cotyledon and embryo. Following the end of the larval and pupal stages (within the seeds), an exit hole is created on the surface of the seed through which the adult emerges (Uyi and Igbinoba, 2016). This sequence of activities brings about a significant reduction in the appearance, viability, marketability, quality and quantity of the cowpea seeds, consequently, threatening food availability and security (Akinkurolere, 2007; Iloba *et al*., 2016).

With respect to pest control, one of the plants used is *Jatropha curcas*, a drought-resistant multipurpose perennial shrub that thrives in the tropics and subtropics of Africa and Asia, but native to the Americas (Sharma, 2017). While several studies have majorly focused on the insecticidal, repellent, anti-feedant and anti-ovipositional activities of the seed oil of *J. curcas* (Adebowale and Adedire, 2006; Bashir and El Shafie, 2013; Sabbour and Abd-El-Raheem, 2013; Abdoul *et al*., 2014), studies on the molluscicidal, nematicidal, fungicidal, antimicrobial and antihelminthic activities of the leaf, seed, and/or stem extracts of *J. curcas* are not uncommon (Liu *et al*., 1997; Sharma and Trivedi, 2002; Igbinosa *et al*., 2009; Juliet *et al*., 2012; Ratnadass and Wink, 2012; Bassem *et al*., 2014). Studies simultaneously focusing on both the insecticidal and anti-ovipositional activities of the leaf powder of *J. curcas* against *C. maculatus* are receiving attention by researchers (Adebowale and Adedire, 2006; Sharma, 2017). Therefore, the objective of this paper is to investigate the insecticidal and anti-ovipositional activities of the leaf powder of *J. curcas* against *C. maculatus*.

MATERIALS AND METHODS

Collection and Preparation of Plant Powders: Fresh leaves of *J. curcas* were collected locally in and around Uwasota area in Benin City (6°22′50″N, 5°35′52″E), Nigeria. Following collection, the leaves were cleaned, chopped and air-dried in the room to a constant weight. The dried leaves were blended into fine powder using electric blender. The powder was sieved with a muslin cloth for homogeneity. The relatively homogenous powder was then preserved in an air-tight and water-proof container pending use.

Insect Culture: The cowpea seed beetle, *C. maculatus* used to establish the insect culture was obtained from infested cowpea seeds purchased locally from Uselu market in Benin City, Nigeria and the culture of *C. maculatus* was raised in the laboratory of the Department of Animal and Environmental Biology, University of Benin, Benin City, Nigeria. Adult *C. maculatus* (males and females) were separated from the infested cowpea seeds, thereafter, introduced into three 4-litre aerated plastic containers (with a perforated screw top lid) containing non-perforated, healthy and insectfree cowpea seeds. Containers (with adult beetles) were kept for six days in the laboratory for mating and oviposition. Following mating and oviposition, adult beetles were removed from the containers and the eggs laid on the seeds were allowed to develop. The plastic containers were left undisturbed until the emergence of the adult beetles. Freshly emerged F1 generation adults were used for the experiments.

Insect Mortality and Oviposition Bioassays: Clean and un-infested cowpea seeds were examined by physical observation for any existing eggs or larvae and thereafter the cowpea seeds were placed in airtight plastic bags ((60 x 30 cm) which were then placed into a deep freezer for 48 hours to ensure that all life stages of the beetle died (Obeng-Ofori et *al.,* 1997).

Different quantities of the leaf powder of *J. curcas* viz. 1.0, 2.0 and 3.0 g were weighed and each added to 20 g of clean undamaged and un-infested cowpea seeds in transparent 300 ml plastic containers. The cowpea seeds in the controls contained no plant powder. The containers with their contents were gently shaken to ensure thorough admixture after which five pairs of three-day-old sexed adults of *C. maculatus* were introduced into each container. All treatments including the control were replicated five times. Adult mortality in all treatments was observed and recorded at 24, 48, 72, 96 and 120 hours. The insects were considered dead when they failed to respond to touch (gentle probing of the abdomen with a pin). Dead adults were removed at each assessment, counted and recorded.

Anti-ovipositional activity was assessed by taking the counts of the number of eggs laid on the grains in treated and control containers (using a hand lens) after 7 days of mating and oviposition. Percentage of oviposition deterrence was calculated according to Singh (2011) using the formula. Percentage of OD = number of eggs laid on control seeds – number of eggs laid on treated seeds ÷ number of eggs laid on control seeds x 100, where OD = oviposition deterrence.

Statistical Analysis: The effects of three concentrations of *J. curcas* leaf powder on the mortality and oviposition rate of *C. maculatus* were analyzed using analysis of variance (ANOVA). The differences among significant treatment means were separated using Turkey's Honest Significant Difference (HSD). The relationships between biopesticide exposure time and insect mortality for all concentrations were established using regression analysis. With the exception of the regression analysis that were performed using Genstat 12.0 (VSN International Limited, United Kingdom) all other analysis were performed using SPSS statistical software, version 20.0 (SPSS, Chicago, USA).

RESULTS

Mortality of Beetles: The leaf powder of *J. curcas* exhibited varying levels of mortality against *C. maculatus* compared to the control (Figures 1 – 5). Following a 24 hour exposure period of *C. maculatus* to different treatment levels (1.0, 2.0 and 3.0 g) of *J. curcas* leaf powder, percentage mortality differed significantly ($F_{3,19}$ = 20.69, p<0.001) (Figure 1). In contrast, percentage mortality of *C. maculatus* did not differ ($F_{3,19}$ = 45.32, p>0.001) as a function of the different concentrations of the leaf powder, when exposed for 48 hours (Figure 2). In the 72 hour exposure period, the concentration of the leaf powder had no significant effect on the number of dead beetles ($F_{3,19}$ = 65.16; p>0.001) as all concentrations (1.0, 2.0 and 3.0 g) demonstrated significant insecticidal activity (86 – 90 %) against the bruchid beetle (Figure 3). Following a 96 hour exposure period of *C. maculatus* to different concentrations (1.0, 2.0 and 3.0 g) of *J. curcas* leaf powder, the percentage mortality of the bruchid beetle did not differ ($F_{3,19}$ = 168.76, p>0.001) according to the concentrations of the leaf powder used (Figure 4) with the 1.0 g treatment demonstrating the highest mortality (98%) followed by 2.0 and 3.0 g treatments both of which caused 92 % mortality respectively.

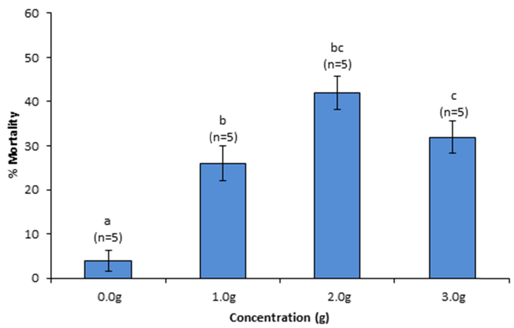

Figure 1: Effects of different concentrations of the leaf powder of *Jatropha curcas* on the percentage mortality of *Callosobruchus maculatus* following a 24-hour exposure period. *Means capped with different letters are significantly different (after Tukey's Honest Significant Difference (HSD) test: $p<0.05$). Sample sizes are given in parenthesis*

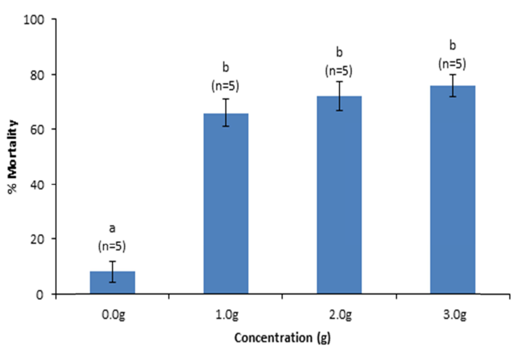

Figure 2: Effects of different concentrations of the leaf powder of *Jatropha curcas* on the percentage mortality of *Callosobruchus maculatus* following a 48-hour exposure period. *Means capped with different letters are significantly different (after Tukey's Honest Significant Difference (HSD) test: $p<0.05$). Sample sizes are given in parenthesis*

Similarly, after exposing the bruchid beetles to different concentrations of *J. curcas* leaf powder for 120 hours, percentage mortality did not differ significantly according to concentration ($F_{3,19}$ = 123.28, $p<0.001$) (Figure 5) with the 1.0 g treatment evoking the highest mortality (98 %) followed by 2.0 and 3.0 g treatments causing 96 and 94 % mortalities of *C. maculatus* respectively. The regression analysis revealed that percentage mortality of *C. maculatus* increased with an increase in the exposure period of the pest to the leaf powder of *J. curcas* (Figure 6).

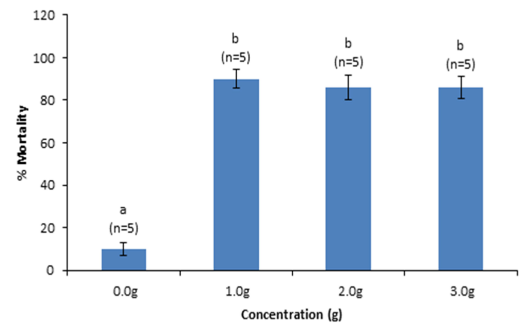

Figure 3: Effects of different concentrations of the leaf powder of *Jatropha curcas* on the percentage mortality of *Callosobruchus maculatus* following a 72-hour exposure period. *Means capped with different letters are significantly different (after Tukey's Honest Significant Difference (HSD) test: $p<0.05$). Sample sizes are given in parenthesis*

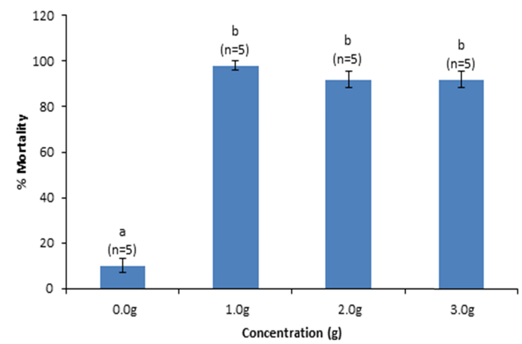

Figure 4: Effects of different concentrations of the leaf powder of *Jatropha curcas* on the percentage mortality of *Callosobruchus maculatus* following a 96-hour exposure period. *Means capped with different letters are significantly different (after Tukey's Honest Significant Difference (HSD) test: $p<0.05$). Sample sizes are given in parenthesis*

Oviposition Rate: The mean oviposition rate by *C. maculatus* on seeds treated with *J. curcas* leaf powder was significantly different ($F_{3,19}$ = 5.57; p = 0.008) from oviposition on the untreated control five days after infestation (Figure 7). At concentrations of 1.0 and 3.0 g of *J. curcas* leaf powder, there was no significant difference in the mean oviposition (Figure 7). The percentage of oviposition deterrence of the leaf powder at 1.0, 2.0 and 3.0 g concentrations were 81.04, 62.58 and 73.15 % respectively.

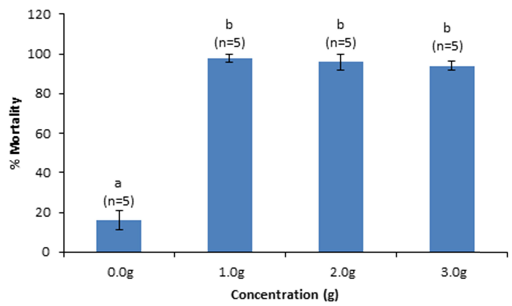

Figure 5: Effects of different concentrations of the leaf powder of *Jatropha curcas* on the percentage mortality of *Callosobruchus maculatus* following a 120-hour exposure period. *Means capped with different letters are significantly different (after Tukey's Honest Significant Difference (HSD) test: p<0.05). Sample sizes are given in parenthesis*

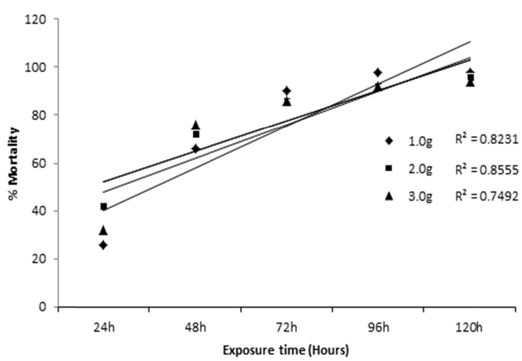

Figure 6: The relationships between the percentage mortality of *Callosobruchus maculatus* and exposure duration at different concentrations of the leaf powder of *Jatropha curcas*

DISCUSSION

This study investigated the insecticidal and anti-ovipositional activities of the leaf powder of *J. curcas* against the cowpea beetle, *C. maculatus*. Results from this study revealed that the leaf powder of *J. curcas* possesses significant insecticidal and anti-ovipositional activities against the *C. maculatus*. Several studies have consistently reported the insecticidal activities of the seed oil, leaf, stem and/or seed extracts of *J. curcas* against several insect pests including *C. maculatus* (Adebowale and Adedire, 2006; Bashir and El Shafie, 2013; Sabbour and Abd-El-Raheem, 2013; Abdoul *et al*., 2014).

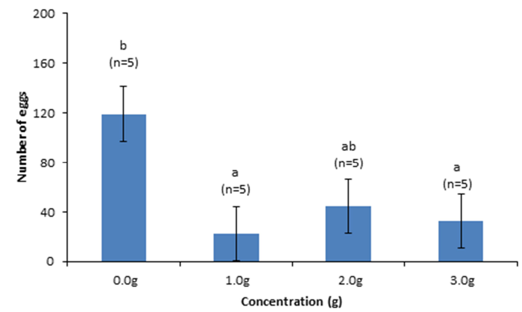

Figure 7: Oviposition rate of *Callosobruchus maculatus* at different treatment levels of *Jatropha curcas* leaf powder following 5 days after infestation (DAI). *Means capped with the same letters are not significantly different (Tukey's Honestly Significant Difference [HSD] test: p>0.05)*

Studies on the effect of the leaf powder of *J. curcas* on oviposition in *C. maculatus* are scanty (Suleiman *et al*., 2014). In this study, the leaf powder of *J. curcas* demonstrated excellent insecticidal activity against adult *C. maculatus* at all tested concentrations. This result corroborated the findings of Olufumilayo (2015), Ofuya *et al*. (2015) and Iloba *et al*. (2016) who reported high mortalities of *C. maculatus* at all tested concentrations of the botanicals used. At the lowest concentration, the leaf powder of *J. curcas* caused above 90 % mortality of *C. maculatus* after 120-hour exposure duration. In agreement with studies of Musa *et al*. (2009), Kouninki *et al.* (2010), Ojo and Ogunleye (2014) and Iloba *et al*. (2016) there was duration dependent potency of *J. curcas* leaf powder against *C. maculatus* with increase in exposure period resulting in more mortalities.

Studies focusing on the anti-ovipositional activities of the seed oil, and/or seed extracts of *J. curcas* are common (Adebowale and Adedire, 2006), nevertheless, studies on the anti-ovipositional activities of the leaf powder of *J. curcas* are scanty. In this study, the leaf powder of *J. curcas* demonstrated considerable anti-ovipositional activities against *C. maculatus* across all concentrations. The fact that the leaf powder of *J. curcas* at all tested concentrations (1.0 – 3.0 g) exhibited excellent insecticidal and anti-ovipositional activities against *C. maculatus*, not only indicated the efficacy of *J. curcas* leaf powder at lower concentrations, but also suggested the presence of several bioactive compounds (phytochemicals) in the leaves of the plant. Although in this study, the phytochemical composition of *J. curcas* was not studied; Mgbemena *et al*. (2016) had earlier reported the presence of phytochemicals such as saponins, tannins, alkaloids, flavonoids and cyanogenic glycosides in *J. curcas.* These compounds have been reported to demonstrate insecticidal, repellent, anti-molting, herbicidal, nematicidal, fungicidal and antimicrobial activities against diverse insects, pathogens and weeds (Al-Rajhy *et al*., 2003; Thoden *et al*., 2009; Chaieb, 2010; Agaba and Fawole, 2014). In conclusion, this study clearly demonstrates that the leaf powder of *J. curcas* can be used as a sustainable substitute to conventional insecticides in the control of *C. maculatus* in stored products in Nigeria and elsewhere.

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