PRINT ISSN 1119-8362
Electronic ISSN 1119-8362

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://ww.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 24 (4) 645- 652 April 2020

Phytochemical Constituents and Insecticidal Efficacy of the Root and Leaf Powders of *Mimosa diplotricha* and *Aspilia africana* against *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae)

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ABSTRACT: This study investigated the efficacy of the root and leaf powders of *Aspilia africana* and *Mimosa diplotricha* against the cowpea beetle, *Callosobruchus maculatus*. Newly emerged adults of *C. maculatus* were exposed to grains treated with one of four treatments (powders: 0.5, 1.0, 1.5 and 2.0 g) of the two plants at different exposure period of 24, 48, 72 and 96 hours. Both *A. africana* and *M. diplotricha* exhibited a high level of mortality which was a function of treatment concentration and of exposure time. At 96 hours of exposure, 2 g of the root and leaf powders of *A. africana* caused 70% and 54% mortality respectively, in *C. maculatus*. When *C. maculatus* was exposed to 2 g for a 96-hour exposure, the root and leaf powders of *M. diplotricha*, however, resulted in 52% and 50% mortality respectively. Although powders from all four treatment types exhibited insecticidal activities by causing varying levels of mortality in *C. maculatus*, the highest death rate was caused by the root powder of *A. africana*. Qualitative analysis of the plants revealed that alkaloids, flavonoids, saponins, steroids, tannins, terpenoids and glycosides were present in the leaf extracts of *A. africana* and *M. diplotricha*. The moderately high insecticidal activity demonstrated by the root powders suggest that they hold more potential in the control of *C. maculatus* compared to the leaf powders.

DOI: https://dx.doi.org/10.4314/jasem.v24i4.16

Dates: Received: 11 February 2020; Revised: 14 March 2020; Accepted: 22 March 2020

Keywords: *Mimosa diplotricha*, *Aspilia africana*, phytochemical constituents, *Callosobruchus maculatus*

The increased and persistent usage of synthesized insecticides in the control of pests have created a lot of ecological as well as environmental challenges. The use of pesticides pose a big threat to food security and human existence (Ghosh *et al*., 2007), thus, there is need to develop an environmental friendly alternative that would help combat this challenge while maintaining a balance in the flora and fauna of the ecosystem (Akinneye *et al*., 2006). *Vigna unguiculata* (L) (Fabaceae), commonly known as cowpea, is a highly profitable crop of economic importance in Nigeria (Abba, 2013) where it is usually and commonly referred to as 'beans'. It is a very nutritious crop that serves as a good and cheap source of protein. The usefulness of cowpea to Nigerians cannot be over emphasized as all parts of the plant are fully utilized to serve many purposes. Like other grain crops, *V. unguiculata* is not free from pest attacks. The cowpea bruchid, *Callosobruchus maculatus* (Fab.) (Coleoptera, Chrysomelidae) is one of the most notorious pests of cowpea (Deshpande *et al*., 2011). It is reported to be the most destructive pest in storage (Deshpande *et al*., 2011) due to the massive destruction caused to seeds and losses in yield (NARO, 2012). Several attempts have been made to control the effects of *C. maculatus,* most of which have been with the use of synthetic pesticides (Agaba *et al*., 2015). The ineffectiveness and the several negative effects of the chemical control measures employed thus far has prompted the search for a sustainable method of controlling these pests and a divergence into the virtually surrealistic world of botanicals and their uses in pest control (Mishra, 2013; Onunkun, 2013) since it leaves no harmful trace in the environment. Botanicals involve the utilization of plant extracts such as, essential oils or powders in insect pest control. Although this appears to be a better prospect in pest control, the efficacy of its use only has little empirical evidence (Udebuani *et al*., 2015; Uyi and Igbinoba, 2016), even though there exists studies on the insecticidal properties of plants (Uyi and Obi, 2017; Opuba *et al.*, 2018; Uyi and Samugana, 2018).

The giant sensitive plant, *Mimosa diplotricha* C. Wright ex Sauvalle (Mimosaceae) and *Aspilia Africana* (Pers.) C. D. Adams (Astaraceae) were both used for this study. *Mimosa diplotricha* is a leguminous shrub native to the Neotropics (Holm *et*

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al., 1977; Waterhouse and Norris, 1987) and invasive to Africa. Its presence in Nigeria dates back to the early or late 1980s (Uyi, 2020). It is an annual and fast growing scrambling but erect shrub that can form dense and problematic thickets within a short period of time (Uyi, 2020) while seriously competing with indigenous vegetation (Lockett and Albin, 1990). It has the potential of acclimatizing quickly and adapting to any weather condition as well as *p*H range (Ekhator *et al.,* 2013; Uyi, 2020). However, *A. africana* is indigenous to Africa and grows extensively across the tropics (Dalziel, 1973). It is used extensively in traditional medicine to treat a wide variety of ailments and health related issues due to its high medicinal value (Elufioye and Agbedahunsi, 2004; Moronkola *et al*., 2007). In Nigeria, the leaves of *A. africana* has been used in treating malaria , *Diabetes mellitus*, sore throat, liver and menstrual pains (Elufioye and Agbedahunsi, 2004; Moronkola *et al*., 2007), tuberculosis, corneal opacities, stomach disorders, haemostatic and skin rashes (Olowokudejo *et al.,* 2008) and cleaning wounds (Okoli *et al*., 2007).

Although studies have shown that certain plants such as *Chromolaena odorata* (L.) King and Robinson (Asteraceae) and *Jatropha curcas* (L.) (Euphorbiaceae) possess repellent and insecticidal properties (Uyi and Igbinoba, 2016; Uyi and Obi, 2017; Opuba *et al*., 2018), however, recent studies (Uyi and Samugana, 2018; Uyi *et al*., 2018) have reported the repellent and insecticidal properties of *M. diplotricha* extracts and powders but studies on the insecticidal activities of *A. africana* are scarce or non-existent. This study, therefore, investigated the phytochemical constituents and insecticidal activities of the root and leaf powders of *M. diplotricha* and *A. africana,* against *C. maculatus*.

MATERIALS AND METHODS

Insect culture: Infested and un-infested cowpea seeds were purchased from New Benin market, Benin City, Edo State, Nigeria. While the un-infested cowpea seeds were freeze dried, pending further use, infested seeds were transferred to a 4-litre plastic container and kept in the laboratory. From the above, a culture of *C. maculatus* was grown in the laboratory at an ambient temperature of 25 ± 4 ˚C and the adults were separated from the cowpea seeds, after the occurrence of mating and oviposition had been confirmed. The cowpea seeds were placed in clean transparent but aerated containers and left undisturbed in the laboratory pending the hatching of oviposited eggs and their use for the trials.

Collection and preparation of plant powders: Mimosa diplotricha and *A. africana* plants were collected from an overgrown farmland at Ekosodin Community, Ovia North East Local Government Area, Edo State, and from a sparse vegetation at the Faculty of Life Sciences, University of Benin, Benin City, (6˚23'N, 5˚61'E), respectively. They were identified by Taxonomists from the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. After identification, the leaves and roots were chopped off and rinsed in water before being air dried for 15 days at room temperatures ranging between 25˚C to 30˚C. The dried plant parts were blended into fine particles using an electric blender and then stored in an airtight and water-proofed container pending further use.

Mortality bioassay: In performing the mortality bioassay, four different treatment types; roots and leaves of *A. africana* and roots and leaves of *M. diplotricha* were used and the experiment was performed at an ambient temperature of 23 ± 4˚C and a relative humidity of 80 ± 5 % at the laboratory of the Department of Animal and Environmental Biology, University of Benin, Benin City, Nigeria. Different quantities of these plants powders (0.5 g, 1.0 g, 1.5 g and 2.0 g) were weighed using a AWS SC-2KGA sensitive balance and added to 20 g of un-infested cowpea seeds placed in properly labeled 100 ml plastic containers (commonly known as disposable cups) and then sealed up with aluminum foil held tightly in place with rubber band. This was thoroughly shaken to ensure that the seeds mix up with the treatment. No treatment was administered to containers meant for control. Using a blade, a small rectangular incision was made at the top of the aluminum foil through which, ten pairs of 1-3 days old sexed but unmated adults of *C. maculatus* were introduced into the containers. The openings were carefully sealed with masking tape and the treatments, including the control, were replicated five times. The containers, including controls, were arranged in a completely randomized pattern. The insect mortality was counted and noted in a properly prepared data sheet at 24, 48, 72 and 96 hours, after insects were confirmed dead when they do not respond to probing with a soft brush. After each counting, dead insects were removed from each container and discarded.

Phytochemical analysis of plant material: 5 g of plants samples were subjected through qualitative phytochemical analysis using methods reported in Keay *et al*. (1964) and Ejikeme *et al*. (2014).

Test for alkaloids (dragendroff test): To 1 ml of methanolic extract in two different test tubes, 2-3 drops of Dragendroff's and Meyer's reagents were added into the different test tubes. The presence of orange red precipitate/turbidity with dragendroff's reagent or white precipitate with Meyer's reagent inferred the presence of alkaloids.

Test for flavonoids (magnesium ribbon test): To 4 ml of the plant extract, a piece of magnesium ribbon was added followed by few drops of concentrated HCl. The presence of color ranging from crimson to magenta indicated that flavonoids are present.

Test for glycosides (Keller Killianoi test): Keller Killianoi method was used to test for the presence of glycosides. To 2ml of plant extract, 1ml of glacial acetic acid with Iron (III) chloride and conc. H_2SO_4 was added. The appearance of blue color was observed indicating the presence of glycosides.

Test for saponins (frothing test): 1 ml of plant extract was measured in a test tube and 5ml of distilled water was added and vigorously shaken. A persistent froth that lasted for at least 15 minutes was observed and this formed an emulsion when few drops of olive oil was added, confirming the presence of saponins.

Test for tannins (ferric chloride test): 2 ml of the extract diluted with distilled water was measured into separate test tubes and to these was added 2-3 drops of 5% Iron (III) chloride ($FeCl_3$) solution. A green-black or blue-black coloration indicated the presence of tannins.

Test for terpenoids: A mixture of 2ml chloroform and 3ml concentrated H_2SO_4 acid was added to 5ml of each extract to form a layer. The presence of a reddish-brown coloration at the interface shows positive results for the presence of terpenoids.

Test for steroids: To 2ml of the plant extract, 1ml of concentrated H_2SO_4 was added carefully along the sides of the test tube. A red color produced in the chloroform layer infers the presence of steroids.

Test for phenols: Phenols were determined spectrophotometrically using 2.0ml of the plant extract.

Statistical analysis: The percentage mortality effects of control treatment and four concentrations (0.5 g, 1.0 g, 1.5 g and 2.0 g) of different plant parts (leaf and stem) powders of *M. diplotricha* and *A. africana* on *C. maculatus* 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 treatment means were compared using the Tukey's Honest Significant Difference (HSD) test. All data were analyzed using SPSS Statistical software, version 16.0 (SPSS, Chicago, USA).

RESULTS AND DISCUSSION

Mortality bioassay: The leaf and root powders of *M. diplotricha* exhibited insecticidal activities by causing varying levels of mortality to *C. maculatus* (Figures 1a-d). When *C. maculatus* was exposed to different concentrations (0.5 g, 1.0 g, 1.5 g and 2.0 g) of the leaf and root powders of *M. diplotricha* for 24 hours, percentage mortality differed as a function of concentration ($F_{4,40}$=7.83; P=0.001) but not as a function of plant part ($F_{4,40}$=1.75; P=0.193) (Figure 1a). The 0.5 g treatment caused the least mortality (8 and 12% for the leaf and root powders, respectively) while the 1.0 g, 1.5 g and 2.0 g caused high mortality (14-24%) but the difference was not significant between the three treatments (Figure 1a). Similarly, beetle mortality differed as a function of concentration ($F_{4,40}$=8.64; P=0.001) but not as a function of plant part ($F_{4,40}$=0.74; P=0.394) when the insects were exposed to different concentrations of the leaf and root powders of *M. diplotricha* for 48 hours (Figure 1b). Apart from the control treatment that caused the least mortality (8-10 %), the 0.5 g treatment caused low mortality (18 and 24% for leaf and root, respectively) while the 1.0 g, 1.5 g and 2.0 g caused high mortality (26-32%) (Figure 1b). When *C. maculatus* was exposed to different concentrations of the leaf and root powders of *M. diplotricha* for 72 hours, percentage mortality differed as a function of concentration ($F_{4,40}$=13.65; P=0.001) but not as a function of plant part ($F_{4,40}$=0.55; P=0.463) (Figure 1c). Apart from the control treatment that caused the least mortality (12%), the 0.5 g treatment caused low mortality (24 and 28% for leaf and root, respectively) while the 1.0 g, 1.5 g and 2.0 g caused high mortality (36-46%) but the difference was not significant between the three treatments (Figure 1c). Similarly, beetle mortality differed as a function of concentration ($F_{4,40}$=22.03; P=0.001) but did not differ as a function of plant part ($F_{4,40}$=2.5; P=0.121) when the insects were exposed to different concentrations of the leaf and root powders of *M. diplotricha* for 96 hours (Figure 1d). Apart from the control treatment that caused the least mortality (12%), the 0.5 g treatment caused low mortality (26 and 32% for leaf and root, respectively) while the 1.0 g, 1.5 g and 2.0 g caused high mortality (40-52%) (Figure 1d). The leaf and root powders of *A. africana*

exhibited insecticidal activities by causing varying levels of mortality to *C. maculatus* (Figure 2a-d).

When *C. maculatus* was exposed to different concentrations of the leaf and root powders of *A. africana* for 24 hours, percentage mortality differed as a function of concentration ($F_{4,40}$=7.71; P=0.001) but not as a function of plant part ($F_{4,40}$=0.04; P=0.834) (Figure 2a).. Beetle mortality differed as a function of concentration ($F_{4,\ 40}$=41.22; P=0.001) and as a function of plant part ($F_{4,\ 40}$=24.00; P=0.001) when the insects were exposed to different concentrations of the leaf and root powders of *A. africana* for 48 hours with the root powder causing higher mortality compared to the leaf powder (Figure 2b).

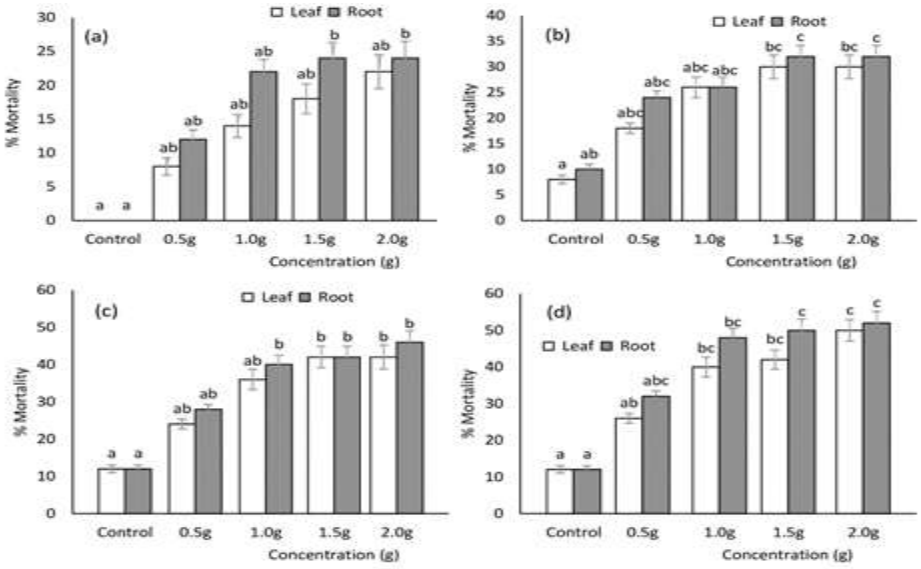

Fig 1: Effects of different concentrations of the leaf and root powders of *Mimosa diplotricha* on the percentage mortality (mean ± se) of *Callosobruchus maculatus* (a) 24-hours (b) 48-hours (c) 72-hours (d) 96-hours. Means capped with different letters are significantly different (after Tukey's Honest Significant Difference [HSD] test: $P < 0.05$).

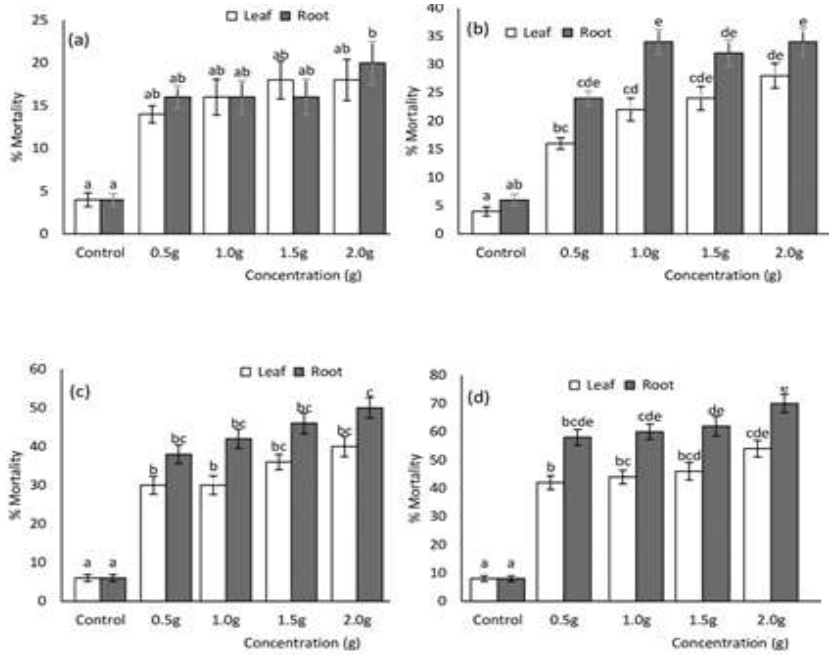

Fig 2: Effects of different concentrations of the leaf and root powders of *Aspilia africana* on the percentage mortality (mean ± se) of *Callosobruchus maculatus* (a) 24-hours (b) 48-hours (c) 72-hours (d) 92-hours. Means capped with different letters are significantly different (after Tukey's Honest Significant Difference [HSD] test: $P < 0.05$).

The 2.0 g treatment recorded the highest mortality (28 and 34% for leaf and root powders, respectively) while the control treatment accounted for the least mortality (4-6% mortality) (Figure 2b). On 72-hours exposure of *C. maculatus* to different concentrations of the leaf and root powders of *A. africana*, mortality differed significantly as a function of concentration ($F_{4, 40}$=31.09; P=0.001) and as a function of plant part ($F_{4, 40}$=10.53; P=0.002). While control treatment caused the lowest mortality (6% each of leaf and root), 0.5 g, 1.0 g, 1.5 g and 2.0 g resulted in high mortality even though the difference was not significant between the three treatments (30-50%), with the root powder causing the highest mortality (Figure 2c).

Likewise, a 96 hours exposure of *C. maculatus* to leaf and root powder of *A. africana* at different concentrations produced mortality that differed significantly both as a function of concentration ($F_{4, 40}$=76.81; P=0.001) as well as plant part ($F_{4, 40}$=34.71; P=0.001) (Figure 2d). Treatment concentrations 0.5 g, 1.0 g, 1.5 g and 2.0 g resulted in high levels of mortality (42-70%), the control treatment, however, only caused 8% mortality both from leaf and root actions. When mortality rate was compared between leaf and root powders of *M. diplotricha* and *A. africana* at 2.0 g treatment concentration in the 96 hours exposure trial, there was significant differences that differed according to plant species ($F_{1, 40}$=9.68; P=0.007) and plant part ($F_{1, 40}$=76.81; P=0.022). Consequently, root powder of *A. africana* caused the highest mortality (70%) while the leaf powder of *A. africana* and *M. diplotricha* caused similar levels of mortality (Fig 3).

This study was conducted to investigate the insecticidal activities of the root and leaf powders of *M. diplotricha* and *A. africana,* against *C. maculatus* and also to determine the phytochemical constituents of the plants. The study revealed that powders from roots and leaves of *M. diplotricha* and *A. africana* exhibited some levels of insecticidal activities, although these were a function of both the treatment concentrations and plant parts used. Earlier studies (Uyi *et al*., 2018) have documented the insecticidal activities of extracts from *M. diplotricha* and *C. odorata* on the worker caste of *Macrotermes* species using root extracts. Also, Uyi and Samugana (2018) demonstrated the potentials of using leaf powder of *M. diplotricha* in controlling *Sitophilus zeamais*. Furthermore, the control of *C. maculatus* has been attempted by several researchers (Olufumilayo, 2015; Ofuya *et al*., 2015; Iloba *et al*., 2016; Uyi and Obi, 2017; Opuba *et al*., 2018) using different concentrations of different plant powders and extracts. For example, Uyi and Igbinoba (2016) demonstrated the potency of *C. odorata* in the control of the beetle. The authors reported more than 80% mortality in a 48 hour exposure trial to the root powder of *C. odorata*. Likewise, Opuba *et al*. (2018) demonstrated the toxicological effects of *Jatropha curcas* on *C. maculatus* and recorded more than 90% mortality and a significant alteration in insect ovipositional tendency. As shown in other studies (e.g. Ojo and Ogunleye, 2014; Iloba *et al*., 2016; Opuba *et al*., 2018), this current research demonstrates the relationship between percentage mortality of insect pest, concentration and exposure period to plant materials.

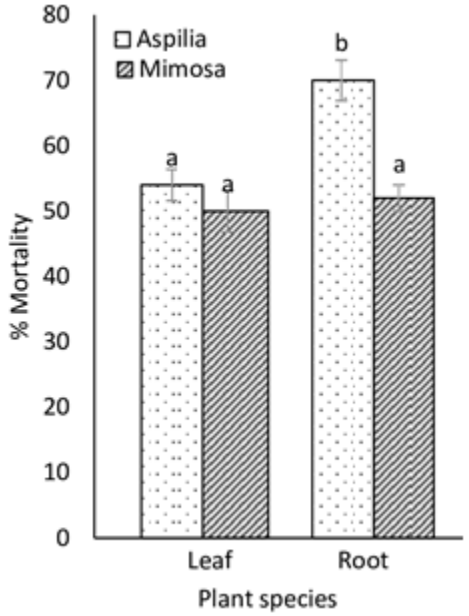

Fig 3: Comparison of effect of 2.0 g of the leaf and root powders of *Aspilia africana* and *Mimosa diplotricha* on the percentage mortality (mean ± se) of *Callosobruchus maculatus* following a 96-hour exposure period. Means capped with different letters are significantly different (after Tukey's Honest Significant Difference [HSD] test: $P < 0.05$).

Similarly, leaf and root powder of *A. africana* resulted in percentage mortality that was dependent on the plant parts and treatment concentrations used. Although the use of *A. africana* in insect pest control has not been documented, the plant has been employed, however, in medicinal purposes (Okoli *et al.,* 2007; Eweka, 2008; Olowokudejo *et al.,* 2008). For instance, Agonihotri *et al.* (2010) reported its use in the treatment of gonorrhea, tuberculosis, cough, rheumatic pains, stomach trouble, corneal opacity, wounds and insect bites. Also, its toxicological effect has been investigated in mice (Oko *et al*., 2011) where it was reported to cause nervous and respiratory disorders. However, studies relating the use of extracts and or powders from *A. africana* in insect pest control are non-existent therefore, this study is the first to report the insecticidal activities of *A. africana*.

In this study, all treatment concentration for both plants demonstrated excellent mortality against the beetle. While leaf of *M. diplotricha* exhibited 50% mortality rate, leaf of *A. africana* showed 54% mortality. On exposure to root powder of *M. diplotricha* and *A. africana*, percentage mortality was 52% and 70%, respectively. Comparatively, it should be noted that in both plants, the highest cause of death was from the root powders even though *A. africana* exhibited a higher mortality than *M. diplotricha*. The mortality recorded from the plants in this study can be attributed to different reasons. Firstly, plant materials are highly fibrous and this translates to the plant powder containing particles that may not be as finely tuned. These particles might block the spiracles of the insect thus leading to asphyxiation (Denloye, 2010). Secondly, plant powders have been known to negatively affect insects by eroding their cuticle and causing dehydration (Kedia *et al*., 2013). Furthermore, Ofuya and Dawodu (2002) reported the impairment of physiological processes by penetration into the insect body via the respiratory or alimentary system. Thirdly, plants are usually characterized by the presence of bioactive phytochemicals which can alter the biochemistry, physiology and metabolism of insects (Udebuani *et al*., 2015).

Table 1: Phytochemical composition of aqueous extracts of leaves and roots of *Aspilia africana* and *Mimosa diplotricha.*

Plant species	Phytochemicals							
	Alkaloids	Flavonoids	Glycosides	Tanins	Saponins	Phenols	Steriods	Terpenoids
A.africana	+	+	+	+	+	+	+	+
M.diplotricha	+	+	+	+	+	+	+	-

Consequently, the phytochemical screening of these plants reveals the presence of secondary metabolites including alkaloids, tannins, saponins, flavonoids, glycosides, steroids, and phenols. These are elemental phytochemicals that gives evidence to the toxicological tendencies of plants. Saponins possess detergent and haemolytic properties that make them highly toxic and enzymatically reactive when injected into the blood stream. Tannins are known to possess astringent and detergent properties as well as inhibitory effects on many enzymes due to protein precipitation (Trease and Evans, 2002). Flavonoids, a class of phenolic compounds, are toxic to insects, fungi, bacteria, nematodes and weeds (Carlsen and Fomsgaard, 2008) because of their antifeeding and deterrent properties.

The alkaloid content of the plants indicate their nematostatic and nematicidal effects on plant-parasitic nematodes (Thoden *et al*., 2009) as alkaloids are well-known as feeding deterrent against herbivores and are toxic for a wide range of non-adapted animals (Thoden *et al*., 2009) while also acting as protease inhibitors (Wen *et al*., 2013). Despite all these plausible reasons, the presence of secondary metabolites holds a better explanation for the observed mortality. However, all of these reasons could be summed up to explain why *A. africana* exhibited a higher level of mortality than *M. diplotricha*. Meanwhile, the potency shown by the root powders of both plants could be the result of a larger concentration of secondary chemicals in them (Biller *et al*., 1994). This is to be expected as roots are the life wire of the plants.

Conclusion: This present study clearly demonstrates the insecticidal activities of the root and leaf powders of *A. africana and M. diplotricha* against *C. maculatus* and suggests that the root powders hold more potential in the control of *C. maculatus* compared to the leaf powders. Therefore our study suggests the usage of powders from both plants as an attractive alternative to synthetic insecticides in the management of *C. maculatus* infestation in Nigeria.

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