Global Scientific JOURNALS

GSJ: Volume 10, Issue 1, January 2022, Online: ISSN 2320-9186

www.globalscientificjournal.com

EXTRACTION OF PHENOLIC COMPOUNDS FROM SODOM APPLE (*Calotropis procera*) AND ITS ROLE IN CONTROL OF LEGUME POD BORER (*Maruca vitrata* FAB) INFESTING COWPEA IN KANO STATE NIGERIA

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ABSTRACTS

Trials were carried out in field cages during 2017 cropping season at teaching and research farm of faculty of Agriculture Bayero University, Kano (11.9836˚N, 8.4753˚E) to evaluate the efficacy of plants phenolic fraction to legume pod borer (Maruca vitrata (G.) Fab). Three cowpea varieties (IT07K-318-33, IT07K-292-10, and IT97K-556-4) were used. Treatments were arranged at Randomized Complete Block Design (RCBD) replicated three times and sprayed at 500µg/ml, 250µg/ml, 125µg/ml, and 0µg/ml (control). Parameters assessed include flower damage, pod damage, and larval mortality. Results of the experiments showed that all concentrations of the treatments were significantly better than control treatments. There was significant difference ($p \geq 0.05$) among the different concentrations and the cowpea varieties. Application of the treatments at 500 and 250µg/ml showed significant reduction of damage due to larval Maruca at both at flowering and the podding when compared with 125 µg/ml and control. Highest protections were recorded in plots sprayed with 500 and 250µg/ml. highest percentage of damage on the pods and flowers were recorded on the untreated control plots. Response of the varieties against the larval damage also varied significantly ($p \geq 0.05$). Highest percentage flower damage was recorded on IT07K-318-33, and then the other varieties, however no significant difference was recorded with respect to pod damage among the three varieties used. Mean percentage mortality was very low on untreated plots which do not differ ($p \geq 0.05$) with 125 µg/ml sprayed plots. Although the calculated LC_{50} was determined at 389.05 µg/ml. Application of the treatments at 500 µg/ml showed highest percentage mortality of the larvae at 66.11% and the mortality tend to reduced to 21.11 % at 250µg/ml.

Key words: *Maruca vitrata*, *Calotropis procera*, *phenolics*,

INTRODUCTION

The legume, cowpea (*Vigna unguiculata* (L) walp) is one of the major food crop grown in many African countries (Egho, 2011). The legume is one of the cheapest source of plant proteins in many diets in tropics and subtropics (Ahmed, Onu, & Mudi, 2015) especially to those who cannot afford animal proteins derived from meat, fish, and eggs (Egho, 2011). Cowpea also provides plant proteins in animal feeds and serves as cover crop important for nitrogen fixation (Ogah, 2013). Grain analyses indicate it contains about 23 -33% proteins, 5-6% fibre, 60-66% carbohydrate, 4.4-3.7% ash and 1.1-3.0% oil (Ahmed et al., 2015).

Nigeria is the world largest cowpea producing Nation (Oparaeke, Dike, & Amatobi, 2005). It is Largely cultivated in the northern part of the country but cultivation recently extend to the southern part where it is grown in the West and East (Egho, Enujeke, & Dialoke, 2014; Egho, 2011). Despite the importance of cowpea, several biotic and abiotic constraints limits its production in the field (Traore, Dabire-basin, Ba, Sanon, & Pittendrigh, 2013). Insects pest are the major biotic constraints to cowpea production, the legume pod borer, *Maruca vitrata* Fab. (Lepidoptera, Crambidae), has been described as the most devastating yield reducing insect pests of cowpea worldwide (Ashigar & Umar, 2016) which can cause typical yield losses ranging from 20% to 80% (Agunbiade *et al.*, 2012; Ogah, 2013; Traore *et al.*, 2013). Adults legume pod borer do not feed on the plant but they mate and lay eggs on them (Rauf & Sadar, 2011). Larvae of *M. vitrata* feed on flowers, stems, peduncles and pods of food legumes, thus damage occurs at all developmental stages from seedling to podding stages. however greatest damage occurs at flowering (Jayasinghe, Premachandra, & Neilson, 2015). To obtain reasonable yield, control of *Maruca vitrata* damage to field crops largely depends upon the timely and application of chemical insecticides, (Ahmed et al., 2015). But success is variable, due to the evolution of resistance to insecticides and the cost of chemical sprays in developing nations (Fernando, Grigolli, Luis, Lourenção, & Ávila, 2015). Furthermore, this practice has resulted in environmental pollution and human health hazard. (Ahmed *et al*., 2015). These reasons make it essentially to switch to other preferable control strategies for the management of these pests. Plants derived extracts have long been studied in attempt to develop alternatives to conventional insecticides but with reduced health and environmental impacts. Santana, et al, 2009 reported phenolic compounds to have substantial applications in agriculture as insecticides.

The Sodom apple *Calotropis procera* is known to contain reasonable amount of phenolics. The crude extracts from this plant have been reported to have insecticidal, repellent, and antifeedant potency against adult and larval insect like *Spodoptera litura*, housefly, culex and aedes mosquitoes (Aktar & Islam, 2015; Bakavathiappan, Baskaran, Pavaraj, & Jeyaparvathi, 2012; Begum, Sharma, & Pandey, 2010; Ibrahim, Martins, Aigbavboa, Gwaram, & Barau, 2017)these potency may likely be as a result of the secondary metabolites with phenolics inclusive. The present study was to evaluate the effects of major phenolic compound from Sodom apple, for the of control of legume pod borer on cowpea.

MATERIALS AND METHODS

Field experiment was conducted at teaching and research farm of the faculty of agriculture, Bayero University, Kano during the 2017 rainy season. Three cowpea varieties viz: IT07K-318-33, IT07K-292-10, and IT97K-556-4 used for the experiment were sourced from IITA Kano. The fields were laid out in randomized complete block design consisting of four treatments at 500, 250, 125 and 0 µg/ml which served as untreated control and each treatment was replicated three times. Each plot was 3.0 x 2.0 m. Seeds were sown at 3 seeds per stand at 2 – 3 cm depth within intra row spacing of 30 cm and inter row spacing of 75 cm apart. Gap filling was done at three weeks after germination to replace dead seedlings. Manual weeding was done at 3 - 4 weeks intervals after planting to ensure clean plots.

Fresh and clean leaves of *C. procera* were collected within Kano metropolis, the leaves were washed and shade dried for two weeks before grinded into fine powder. Extraction of plant materials was done using soxhlex apparatus with 80% ethanol. The extract was then concentrated with rotator evaporator and then subjected to column chromatography using n-hexane, chloroform and methanol as eluting solvents. The fractions were subjected to FTIR analysis for phenolic functionality, the phenolics fraction was then subjected to Gas Chromatography Mass spectrometric (GCMS) analysis for complete and rapid characterization of the fraction identified for phenolics presence. Serial dilution was used to make concentrations of the fraction at 500, 250, 125 and 0 µg/ml which served as untreated control.

Infestation of cowpea flowers by *Maruca vitrata* larvae was assessed at 45 days DAS. Ten flowers were randomly selected from each plot. The cowpea flowers were taken to the laboratory in clean bottle containing 50% alcohol (ethanol) where they were dissected and observed.

Presence of holes, and larvae in a flower was used as index for flower infestation. Observation at 1, 5, and 7 days intervals after spray. The mean score for the 10 flowers was calculated and recorded. Pod damage by *M. vitrata* was assessed in the field by visual observation. Assessment was done at 10 weeks after spray (WAS) when the pods were fully filled and matured but still green. Holes and presence of frass on pods and sticking of pods were used as damage index by *Maruca*. Percentage of pod damage was calculated using the formula according to (Oparaeke et al., 2005).

$$\% \text{ pod damage} = \frac{\text{Total No. of pods produced per plant} - \text{No. of undamaged pods} \times 100}{\text{Total No. of pods produced}}$$

Larval mortality was determined according to Rauf and Sadar 2011, 10 infested pods were randomly picked from each cage and the number of legume pod borer were counted and recorded before spray and after 24 hours after spray to calculate the percentage of larval mortality.

$$\frac{\text{Number of dead larvae} \times 100}{\text{Total Number of larvae}}$$

All data were subjected to analysis of variance (ANOVA) and significant means were separated by Fisher's Least Significant Difference Test (LSD), at 5% level of significance using Minitab 18 statistical software. Probit analysis was used to determine lethal concentration 50 (LC_{50}).

RESULT

In the GC-MS analyses of *C. Procera* , 24 compounds were identified in the chloroform / n-hexane fraction of after column chromatography and fourier transform spectroscopy (FTIR) The identification of phytochemical compounds is based on the peak area (which represents the percentage of that compound), molecular weight and molecular formula. The only phenolic compound identified in the fraction was 2, 4-Di-tert-butylphenol with retention time of 34.6889 has the peak area of 4.18. The Peaks, compound names and retention times of the other compounds are shown in Table 4.1 The total ion chromatograph (TIC) showing the peak identities of the various compounds identified are as shown in Figure 4.1

Table 1: Chemical constituents present in the n-hexane /chloroform fraction using GC-MS analysis.

PK	RT	Area Pct	Names of compounds
1	29.0471	0.2599	1-Hexadecanol
2	33.773	0.9855	Pentadecane
3	34.6889	4.1838	**2,4-Di-tert-butylphenol**
4	36.4474	0.5798	Octatriacontyl pentafluoropropionate
5	37.0335	0.7099	Octatriacontyl trifluoroacetate
6	37.6197	1.2783	Trichloroacetic acid, pentadecyl ester
7	37.9128	2.55	Hexadecane
8	41.906	1.7681	Heptadecane
9	42.0892	2.4232	Hexadecane, 2,6,11,15-tetramethyl-
10	43.8476	0.2939	Cyclododecane, ethyl-
11	45.4229	2.718	1-Octadecene
12	45.716	2.7975	Octadecane
13	46.0091	1.2212	Tetratetracontane
14	46.6685	0.706	Isopropyl myristate
15	47.1448	0.4103	Borane, 2,3-dimethyl-2-butyl- (dimer)
16	47.3646	1.8726	2-Undecanone, 6,10-dimethyl-
17	47.9507	0.6616	Pentadecafluorooctanoic acid, octadecyl ester
18	48.6834	0.8258	Acetic acid, chloro-, hexadecyl ester
19	49.3062	3.1308	Nonadecane
20	50.0756	2.4779	Tetrapentacontane, 1,54-dibromo-
21	51.6142	10.6513	Dibutyl phthalate
22	52.7499	25.472	Eicosane
23	55.5342	2.7487	Heneicosane
24	56.0471	22.6811	Phytol
25	57.1828	6.5931	9,17-Octadecadienal, (Z)-

RT=retention time, PK= peak

Effect of phenolic (2, 4 di-tert-butylphenol) on cowpea flower damage by *M. Vitrata*

Results in table 4.2 shows the percentage of flower damage by *Maruca vitrata* larvae sampled at

50% flowering. The damage caused by *M. vitrata* larvae on flower were significantly ($p<0.05$) reduced for concentrations at 500 and 250 µg/ml sprayed compared with the untreated control, thus there was significant difference ($p<0.05$) between the treatment concentrations, the highest protection was recorded on plots sprayed with 500 (µg/ml) and 250 µg/ml which do not differs significantly, there was no significant difference in plots spray with 125 µg/ml and 0 µg/ml however highest damage was recorded in untreated control plots sprayed with 0µg/ml, similarly there was significant difference for flower damage among the cowpea varieties Fig 1, least damage was recorded on IT97K-556-4 but this do not differs with IT07K-292-10. Highest damage was recorded on IT07K-318-33 which significantly differs from IT97K-556-4, however the two varieties IT07K-318-33 and IT07K-292-10 do not differ significantly.

Table 2: Effects of phenolic (2, 4 di-tert-butylphenol) spray on flower damage by *M. vitrata* larvae at 50% Flowering during 2017 rainy season at Kano, Nigeria.

Tr. No.	Concentration (µg/ml)	*Mean flower damage (%) for the three varieties after spray		
		IT07K-318-33	IT07K-292-10	IT97K-556-4
1	500	4.4 ^{c}	3.3 ^{c}	0.0^{c}
2	250	10 ^{bc}	5.6 ^{bc}	3.3 ^{bc}
3	125	21.1 ^{ab}	13.3 ^{ab}	8.9 ^{ab}
Untreated control	0	24.5 ^{a}	17.8^{a}	11.1^{a}
S.E±	-	3.06	2.46	1.97

Means followed by the same letters within column are not significantly different at $p \leq 0.05$ (LSD). * Means of the three observations for the three replicates.

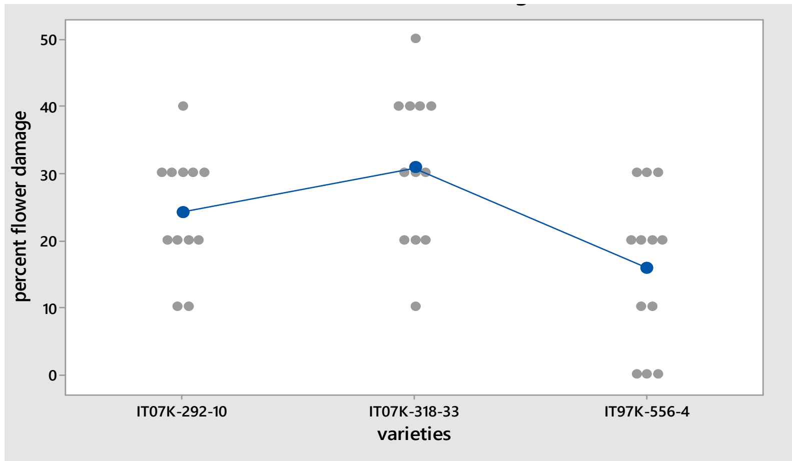

Figure 1: individual value plot for percentage of flower damage vs. varieties. Significantly difference at ($p \leq 0.05$) LSD

Effect of phenolic (2, 4 di-tert-butylphenol) on cowpea pod damage by *M. Vitrata*

Results in table 3 shows the percentage of pod damage caused by *Maruca vitrata* larvae sampled at 60DAP. The damage of the *M. vitrata* larvae were significantly ($p<0.05$) reduced for all concentrations sprayed compared with the untreated control, the highest protection was recorded on cages sprayed with 500 (µg/ml) which do not significantly differs with plots spray with 250 (µg/ml). Higher pod damage was recorded in plot spray with 125(µg/ml) however is record do not differs with plot spray with 250 (µg/ml), control cages with 0 (µg/ml), recorded the highest percentage of pod damage. There were no significant difference ($p<0.05$) on pod damage between the three cowpea varieties. Fig 2.

Table 3:Effect of phenolic (2, 4 di-tert-butylphenol) on cowpea pod damage by *M. Vitrata*

Tr. No.	Concentration (µg/ml)	Mean pod damage (%) for the three varieties		
		IT07K-318-33	IT07K-292-10	IT97K-556-4
1	500	1.94c	2.78c	1.67c
2	250	8.67bc	5.57bc	4.18bc
3	125	15.38b	10.88b	6.36bc
Untreated Control	0	20.31a	28.1a	19.4a
S.E ±	-	3.22	3.29	2.49

Means followed by the same letters within column are not significantly different at $p \leq 0.05$ (LSD).

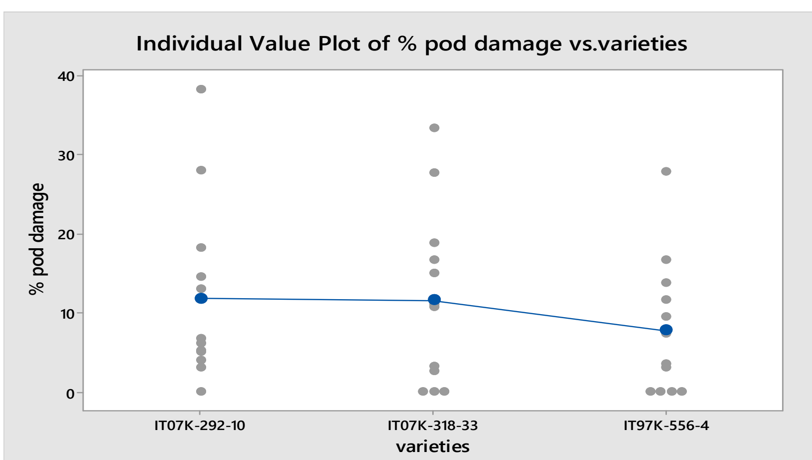

Figure 2: individual value plot for percentage of pod damage vs. varieties. Means do not significantly differs at ($p \leq 0.05$) LSD

Effect of phenolic (2, 4 di-tert-butylphenol) on *M. Vitrata* larval mortality

After 24 hours of the application of the phenolics on the cowpea in the field cages, the highest larvicidal effect of the compounds on the *M. vitrata* was 66% at 500µg/ml which differs statistically ($P \leq 0.05$) with the remaining concentrations Table 4.5. At 250µg/ml the death rate was 24%., the mortality rate at 125µg/ml do not significantly differs with the untreated control. Hence the mortality was very low. The LC_{50} computed for all treatments level was at 389µg/ml.

Table 3: Effect of phenolic (2, 4 di-tert-butylphenol) on *M. Vitrata* larval mortality at 24hours after spray.

Expt.	concentration(µg/ml)	* Mean No. larvae	* Mean No. of dead larvae	*mean% mortality	LC_{50}(µg/ml)
1	500	3.33[a]	2.44 [a]	66.11[a]	
					389.05
2	250	3.56[a]	1.11[b]	26.11[b]	
3	125	3.44[a]	0.22 [c]	4.33 [c]	
Untreated Control	0	3.11[a]	0[c]	0 [c]	

*Average of 3replications, 5 plants in each replication, Means followed by the same letters within columns are not significantly different at $p \leq 0.05$ of fisher LSD test

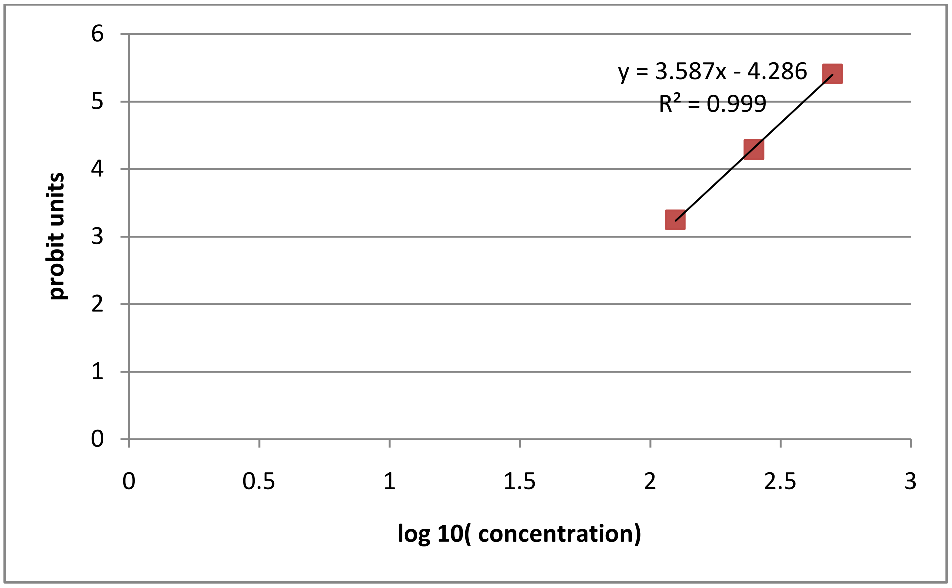

Figure 3: Log-probit regressions of mortality caused by phenol (2, 4 di-tert-butylphenol)

DISCUSSION

Crude extracts of *C. procera* has been reported to have insecticidal and anti feedant properties and there quite effective against certain adult insects and larvae (Rahuman, 2011). The insecticidal potency of this plant emanated from the presence of photochemicals such phenolics, alkaloids, saponins, tannins, flavonoids, anthraquinon and phloboacidtannins (Ibrahim et al., 2017). The study of plant phenolics have been reported to have many practical applications. Many of these phenolics that are physiologically active against herbivores or pathogens are now used as insecticides, fungicides or pharmaceuticals (Ozeker, 1999).

Although there are fewer reports on the effect of *C. procera* extracts on *Maruca*, much research on the effects of different part of this plant has been reported on some larval and insect pest. Similarly previous investigations on *C. procera* reports it insecticidal nematicidal, antimicrobial and antihelminthic activities. It is also effective in the treatment of toothache, cough and subcutaneous diseases. All these investigations mostly utilized the crude extracts of this plant. However only few studies investigated the efficacy of certain bioactive compounds of *C. procera* to determine it insecticidal, nematicidal, antimicrobial, and antihelminthic activities.

The results from the past studies quite indicate the antifeedant property of the different part of *Calotropis* extract which may be due to the different secondary metabolites present in the extract possessing different bioactivities. However not all the bioactive compounds in this plants is effective against insects and in facts the effects of one bioactive may counteracts the effects of another when working with the crude extracts and as a result the actual desirable results may not likely be achieved.

In the present study, the efficacy of phenolic (2, 4 di-tert-butylphenol) from leaves extracts of *C. procera* was investigated. The result from the study revealed that *C. procera* was quite effective in reduction of damages due to *M. vitrata* larvae. The compound significantly reduces pod borer infestation especially when sprayed at 500µg/ml both at flowering and podding stage of the plants however at lower doses the protection effect was less. This result correspond with the work of (Aktar & Islam, 2015; Begum *et al*., 2010) on housefly *Musca domestica*. From the data in these studies, it indicate that the leaf extracts of showed some effects on the house fly and therefore the plant may be utilized as the probable candidates for the development of bioinsecticides to control the population of *Musca domestica* as safer and economic alternatives to the synthetic insecticides.

The findings from the present study on larval *Maruca* mortality due to the applied compound was quiet effective, larval mortality was 66.11% at 500µg/ml, at 250 µg/ml the percentage mortality was 21.1% , this result was not surprising because the effect the ethanol leaves extract of this plant on the third larva instar of the mosquito *Anopheles stephansi* was investigated by Doshi, Satodiya, Thakur, Parabia, and Khan, (2011). In the study, matured extracts of this plant showed high activity against the 3rd instar larvae of the mosquito which exhibited 100% mortality at 2000 ppm after 48 hours of incubation. Similarly Methanolic extracted latex of *C. procera* gave 100% mortality after 1 hour exposure, while water extracted latex gave 60% mortality after 3 hours exposure, this appeared in studying the comparative effectiveness of larvicidal potential of methanol extracted latex of the plant with temephos, a synthetic larvicide which is widely used in all vector control programme against *Aedes aegypti* mosquitoes (Singh, Joshi & Dam, 2005). The whole latex of *Calotropis procera* when the toxicity was evaluated on *Aedes aegypti* upon egg hatching and larval development, also shown to cause 100% mortality of 3rd instars within 5 min. It was fractionated into water-soluble dialyzable (DF) and non-dialyzable (NDF) rubber-free materials. Both fractions were partially effective to prevent egg hatching and most of individuals growing under experimental conditions died before reaching 2nd instars or stayed in 1st instars. On the other hand, the fractions were very toxic to 3rd instars causing 100% mortality within 24h. (Al-snafi, 2015).So also aqueous extract of *C. procera* leaves at 1,000 ppm exhibited 100% larvicidal activity against fourth instar larvae of *Culex tritaeniorhynchus* and *Cx. gelidus.* Extract treatment at 1,000 ppm of both mosquitoes' eggs resulted in to 100% ovicidal activity. At 1,000 ppm, extract provided complete protection from mosquito bite for 240 min against both mosquitoes; however at lower doses the protection time was less (Kumar *et al*., 2012). Similarly the impact of n- hexane leaves extracts of *C. procera* was determined on the Survival, Morphology and Behaviour of Dengue Vector, *Aedes aegypti* L. The larvicidal bioassay conducted with the hexane leaf extract indicates its efficacy revealing the LC50 and LC90 values as 78.39 and 100.60 ppm, respectively. It was also observed from Singh and Arya, (2011) Acetone and Methanol extracts of *C. procera* gave 46.67 and 53.33% larval mortality of Shisham defoliator *(P. reflexa)*, respectively at 1% concentration petroleum ether and distilled water extracts of the plants were not found effective and only provided 20.00 and 10.00% mortality, respectively. Similarly for Poplar defoliator (*C. cupreata)* it was also observed that out of four extracts studied Acetone and methanol extracts were found effective at 1% concentration and gave 40.00 and 50.00% larval mortality. Petroleum ether and distilled water extracts were not found effective and provided only 16.67 and 6.67% larval mortality, respectively.

In the present study, the potency of this compound to reduce pod borer infestation may be likely due to toxic effect against the pod borer or suffocation, antifeedant and or repellent action of the compound. The larvae of the pod borer niches inside the preferred parts of the cowpea plant outside the reach of most insecticides, because of this it is suspected that the compound has been absorbed by the flowers/pods through osmotic pressure which causes the insect to stop feeding, or possibly as the pods absorbed the spray compounds, the soft body of the larvae may have absorbed some of the compound which likely inhibit feeding by the larvae and eventually death. Although the LC_{50} value for all the levels was 389.05µg/ml, however application of the compounds showed more significant effects in larval mortality at 500µg/ml, compared to at 250µg/ml, at 125µg/ml, percentage mortality do not differs with that of the untreated control, however meaning that the higher the concentration the higher the percentage mortality.

The fact in the study that plant phenolics was effective in reducing borer infestation was in agreement with Wójcicka, (2010) who reported that phenolics unfavorably affect growth, development and/or feeding behavior of aphid.

In the present study, three cowpea varieties viz: IT07K-318-33, IT07K-292-10, IT97K-556-4 were used and from the findings, there was no significant difference in the population of *M. vitrata* infestation at the first planting date which may probably be due to the lower population of the *M. vitrata* early in late July to early August, which subsequently built-up as the season progressed. Therefore it is recommended farmers should plant cowpea early to mid

August to avoid late planting which may coincide with the peak period of *M. vitrata* infestation in the field (Musa, 2016) which may be attributed to high humidy and longer vegetative growth phase of the crop because of quite heavy rainfall (Amoah, 2010). These provided a conducive environment for *Maruca* to thrive. Similar results have reported by Musa (2016) and Karungi, Adipala, Ogenga-Latigo, and Kyamanywa (2000) in Uganda.

Some cowpeas are less preferred for feeding, egg layin as well as damage than others. S IT07K-318-33 and IT07K-292-10 in the present study attracted the highest population of *Maruca* larvae on flowers and pods and thus are damaged more compared with IT97K-556-4 were the damage is less. This was attributed to insect pest preference or non preference for some cowpea genotypes which may possess traits that affect the behavior and selection pressure of the insect during search for food, shelter, and oviposition (Onyishi *et al*., 2013). This finding is supported by Stadler (1977) that reported biochemical factors tends to a large extent affect the behavior and metabolic process of pest while morphological factors mostly influence the mechanism of locomotion, feeding, oviposition, ingestion and digestion of pest.

CONCLUSION

The present study shows that plant phenolics at 500µg/ml exhibited insecticidal activity against legume pod borer *M. vitrata* and thus indicating their potentials for development as botanicals for the management of the pest on cowpea plants. The compound did not show did not show any phytotoxicity effect on treated plants and these phenolics have used for long in traditional medicines and now pharmaceuticals and modern medicine for treatment of various ailments and the biodegradable property of th ese materials would also reduce any potential hazard to the environment. Further studies are required to find out the efficacy of other photochemical from this plant materials on *M. vitrata* and other insect pests of cowpea and other cultivated crops and testing their appropriate spraying regimes for effectiveness.

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