

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.

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

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 (LC₅₀).

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 | Area | | Names of compounds |
|----|---------|---------|---|
| | RT | Pct | |
| 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- Pentadecafluorooctanoic acid, octadecyl |
| 17 | 47.9507 | 0.6616 | 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 $\mu\text{g/ml}$ 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 ($\mu\text{g/ml}$) and 250 $\mu\text{g/ml}$ which do not differs significantly, there was no significant difference in plots spray with 125 $\mu\text{g/ml}$ and 0 $\mu\text{g/ml}$ however highest damage was recorded in untreated control plots sprayed with 0 $\mu\text{g/ml}$, 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 ($\mu\text{g/ml}$) | *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 \pm | - | 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.

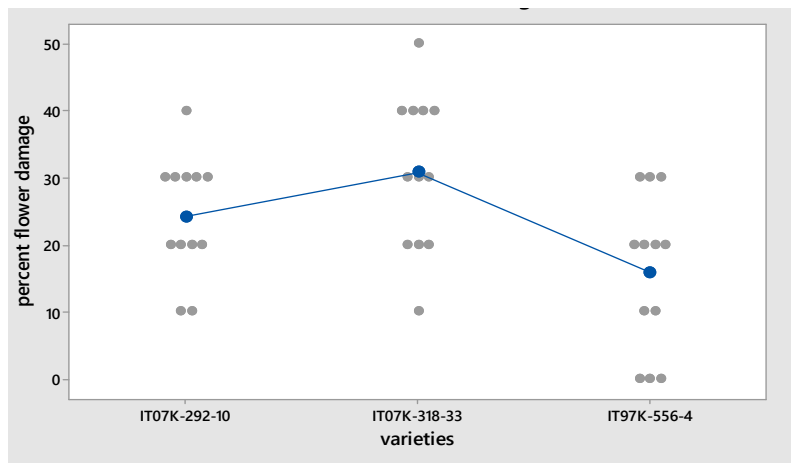


Figure 1: individual value plot for percentage of flower damage vs. varieties. Significantly difference at ($p \leq 0.05$) LSD

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 ($\mu\text{g/ml}$) which do not significantly differs with plots spray with 250 ($\mu\text{g/ml}$). Higher pod damage was recorded in plot spray with 125($\mu\text{g/ml}$) however is record do not differs with plot spray with 250 ($\mu\text{g/ml}$), control cages with 0 ($\mu\text{g/ml}$), 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).

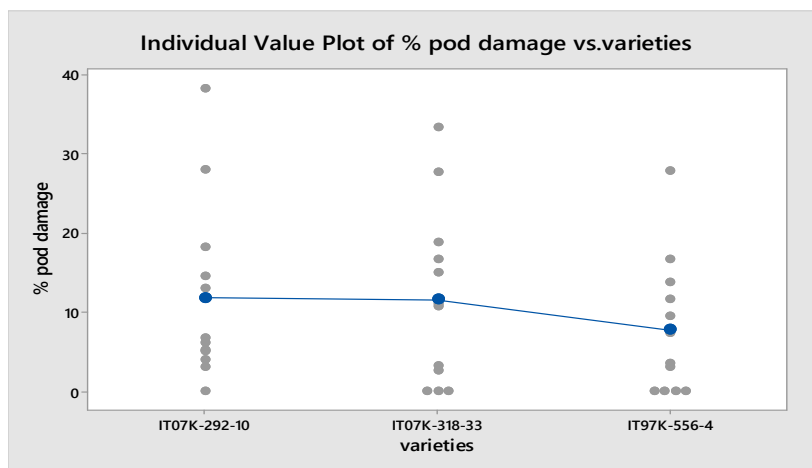


Figure 2: individual value plot for percentage of pod damage vs. varieties. Means do not significantly differs at ($p \leq 0.05$) LSD

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

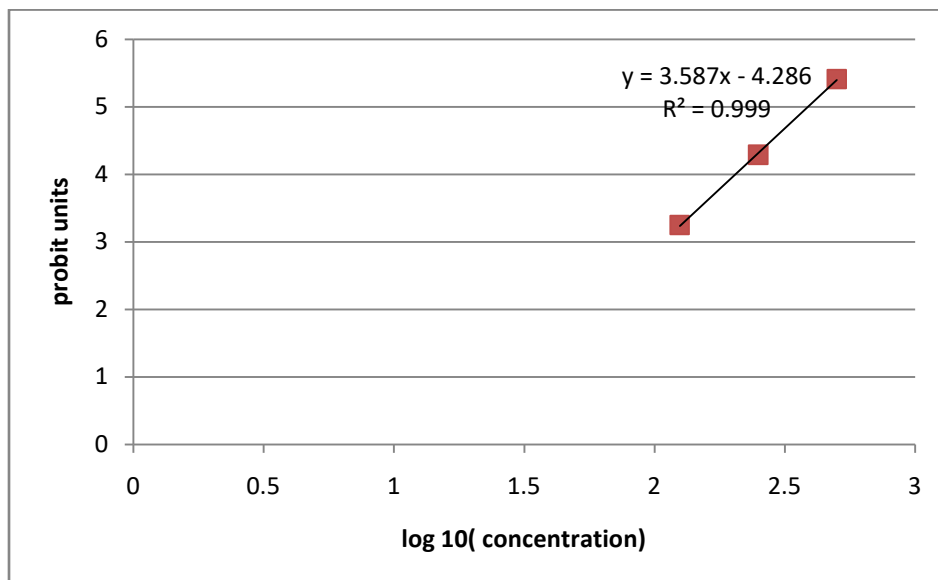


Figure 3: Log-probit regressions of mortality caused by phenol (2, 4 di-tert-butylphenol)

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 nematocidal, 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, nematocidal, 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 LC₅₀ 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 humidity 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 laying 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 where 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 tend 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 any phytotoxicity effect on treated plants and these phenolics have been used for long in traditional medicines and now pharmaceuticals and modern medicine for treatment of various ailments and the biodegradable property of these materials would also reduce any potential hazard to the environment. Further studies are required to find out the efficacy of other phytochemicals 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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