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TOLERANCE TO *PIPER GUINEENSE* (SCHUM AND THONN) POWDER AMONG EKITI STATE POPULATIONS OF *CALLOSOBRUCHUS MACULATUS* (FAB.)

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ABSTRACT

Tolerance to *Piper guineense* (Schum and Thonn) seed powder by populations of *Callosobruchus maculatus* (Fab.) obtained from six major towns spread across Ekiti State, Nigeria was investigated at ambient temperature (28±2ºC) and relative humidity (88±5%). The towns were Ado, Emure, Ijero, Ikere, Omuo and Oye Ekiti. Bruchid samples from each location were exposed to seed powder of *P. guineense* at 0.00, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30g/20g of cowpea seeds. Mortality was assessed at 24, 48, 72 and 96 hours post-treatment. The effects of location, dose and exposure time on the bruchids response were also analysed. The results revealed that bruchid susceptibility to *P. guineense* varied across locations. However, there was significant effect ($p<0.001$) of location, dose and exposure time on bruchid susceptibility to *P. guineense* while significant interactive effects also exist among these factors. At the highest (0.30g) and lowest (0.05g) experimental doses, bruchids obtained from Emure were the most tolerant (LT_{50}: 1.19 hours and 2.45 hours respectively) while those from Ikere were the most susceptible (LT_{50}: 0.62 hours and 1.24 hours respectively) to this botanical. Though, the response of Ekiti State *C. maculatus* populations to *P. guineense* is location dependent, there was no geographical pattern to the tolerance distribution.

Keywords: Botanical, *Callosobruchus maculatus*, mortality, *Piper guineense*, susceptibility.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is one of the most important food legumes in sub-Sahara Africa as it remained the mainstay of the diet of most people. In fact, the crop has continued to be the most cultivated and utilized legume in Nigeria (IITA, 2010; Longe, 2011). Its continuous production is however being hampered by leguminous plants' insect pests such as *Callosobruchus maculatus*. This pest is capable of causing noxious damage on stored cowpea seeds (Karungi *et al*., 1999). Heavy qualitative and quantitative post-harvest losses as high as 90 to 100% on unprotected cowpea seeds after 3-5 months of storage has been reported due to the debilitating effect of this insect pest (Caswell, 1981). The problem is further aggravated by poor and inefficient storage facilities in Nigeria.

The protection of cowpea seeds through pest management has however played a major role in ensuring food security in sub-Sahara Africa. The use of synthetic insecticides remained the most effective method of management especially in large scale production. However, due to public awareness on the myriads of undesirable health and environmental effects associated with most chemical insecticides (Isman, 2008); research studies are now being focused on the use of plant-based insecticides as a wherewithal to most chemical insecticides. Notable among the arrays of entomotoxic botanicals cheaply available in Nigeria is crude seed powder of *Piper guineense* (Schum and Thonn) (African black peeper). This plant is well known for its high efficacy in the protection of cowpea seeds from *C. maculatus* attack (Ofuya and Dawodu, 2001). The *in vivo* insecticidal activity of the plant powder against *C. maculatus* has been compared with synthetic chemicals (Idoko and Adesina, 2012).

In spite of promising results reported on the use of *P. guineense*, detailed study on the potential effect of geographical location on the susceptibility of *C. maculatus* to insecticidal seed powder of this plant remained to be explored. Human activities and environmental conditions, which differ per location, may influence the response of insect population to a particular toxin. These variations could either lessen or augment the ability of most insects to withstand toxins (Gbaye and Holloway, 2011). Consequently, the dosage of a particular chemical required to achieve maximum protection of grains may therefore vary due to ecological differences. Usage of lower or higher dosage of plant material than required by local peasant farmers can lead to botanical resistance overtime (Oyeniyi *et al*., 2015a). This is particularly important considering the high level of illiteracy among indigenous farmers who are responsible for the production of large chunk of cowpea in Nigeria. A comprehensive work is therefore needed to ascertain the aforementioned. Hence, this study investigated the possible impact of location, dose and exposure time on the tolerance among Ekiti State populations of *C. maculatus* to *P. guineense* powder.

MATERIALS AND METHODS

Insect collection and culture

Samples of adult *C. maculatus* from infested cowpea seeds were obtained from retail stores in six major towns that spread across Ekiti State in South-Western Nigeria. The towns were Ado-Ekiti, Emure-Ekiti, Ijero-Ekiti, Ikere-Ekiti, Omuo-Ekiti and Oye-Ekiti. Clean cowpea seeds (Sokoto white cultivar) were disinfested in the freezer at -18^{0}C for two weeks. Prior to use, disinfested cowpea seeds were allowed to equilibrate in the laboratory for three days to prevent mouldiness at ambient temperature and relative humidity (28±2ºC and 88±5%) by placing on laboratory shelf. Two hundred gramme (200g) of disinfested cowpea seeds were weighed into 1.65 litres transparent plastic containers and labelled according to location. Bruchid samples from each location were then introduced into corresponding containers containing already-disinfested cowpea seeds and reared for two generations to reduce maternally inherited dietary effects. The bruchids were reared at ambient conditions stated above.

Preparation of insecticide powder

Dry seeds of *Piper guineense* (African black pepper) were purchased from Oba market, Ilupeju-Ekiti, Ekiti State. They were ground into powder using NAKAI NJ-1731 electric blender. The seed powder was later sifted through a mesh size of 1mm^2 to obtain fine powder and later stored in a plastic container with a tight lid prior to use.

Bruchid susceptibility bioassay

Twenty grammes of cowpea seeds were weighed using a Mettler beam PB 3002 weighing balance into 170ml plastic containers. Plant powder weighing 0.00g (control), 0.05g, 0.10g, 0.15g, 0.20g, 0.25g or 0.30g was added to cowpea seeds and thoroughly mixed inside each plastic container using a glass rod. Freshly emerged twenty five adult *C. maculatus* (0-2 days) were later introduced into each container. Each treatment had four replicates and the experiment was set up in a complete randomized block design. Bruchid mortality was assessed after 24, 48, 72 and 96 hours post-treatment. The procedure was done for samples of bruchid from each of the locations.

Statistical analysis

Abbott (1925) formula was used to correct all data on adult mortality counts using control mortality. The data on adult mortality were checked for normality based on Shapiro-Wilk test before being subjected to one-way analysis of variance (ANOVA) ($p<0.05$) and treatment means were separated using Tukey's test. Data on adult mortality were also subjected to probit analysis (Finney, 1971) to determine the lethal time required by insecticide powder to kill 50% (LT_{50}) of *C. maculatus* from each location. General Linear Model (GLM) was used to determine significant interaction of location, exposure time and dose on the tolerance of *C. maculatus* to *P. guineense*. All analyses were carried out using SPSS 17.0 software package.

RESULTS

Overview

Irrespective of the location, bruchid mortality increased with increasing doses ($F_{6,\ 504}$ =

997.68, $p < 0.001$) of *P. guineense* and exposure time ($F_{3, 504} = 1044.85$, $p < 0.001$). However, *C. maculatus* tolerance to insecticide powder varied from location to location ($F_{5, 504} = 75.13$, $p < 0.001$). There was significant interaction between location versus dose ($F_{30, 504} = 5.17$, $p < 0.001$), location versus exposure time ($F_{15, 504} = 9.20$, $p < 0.001$) and dose versus exposure time ($F_{18, 504} = 31.18$, $p < 0.001$). The overall impact of three way interaction between location, dose and exposure time was also significant ($F_{90, 504} = 1.52$, $p = 0.003$).

Tolerance of diverse populations of *Callosobruchus maculatus* to *Piper guineense* powder

Tables 1-4 show the effect of *P. guineense* powder on the percentage mortality of *C. maculatus* obtained from six geographical locations in Ekiti state after 24, 48, 72 and 96 hours post. Irrespective of the exposure time, mortality of *C. maculatus* populations exposed to each of the tested doses was significantly higher than those exposed to control after 48, 72 and 96 hours post-treatment (Table 2-4). Also, complete bruchid mortality (100%) was observed at the highest experimental doses (0.25g and 0.30g) after 96 hours post-treatment for each population except at 0.25g for Ado population (Table 4). Similarly, mortality of each of the bruchid population exposed at 0.3g after 72 and 96 hours were not significantly different ($p > 0.05$) from each other (Table 3 and 4). However, highest significant effect ($p< 0.001$) of *P. guineense* dose was observed in bruchid obtained from Ikere Ekiti after 48 ($F_{6, 21} = 101.70$), 72 ($F_{6, 21} = 494.32$) and 96 hours ($F_{6, 21} = 5492.31$) while the least significant effect was observed in bruchid samples obtained from Ado after 24 ($F_{6, 21} = 4.46$, $p = 0.005$), 48 ($F_{6, 21} = 59.50$, $p < 0.001$) and 96 hours ($F_{6, 21} = 90.48$, $p < 0.001$) post-treatment.

There was also a significant effect ($p < 0.001$) of *P. guineense* dose on the susceptibility of bruchid from each population (Ado: $F_{6, 84} = 104.45$; Emure: $F_{6, 84} = 180.18$; Ijero: $F_{6, 84} = 160.81$; Ikere: $F_{6, 84} = 212.66$; Omuo: $F_{6, 84} = 219.57$ and Oye: $F_{6, 84} = 176.72$). Likewise, the effect of exposure time on the susceptibility of each population of *C. maculatus* was also significant ($p < 0.001$) (Ado: $F_{3, 84} = 105.00$; Emure: $F_{3, 84} = 362.54$; Ijero: $F_{3, 84} = 210.09$; Ikere: $F_{3, 84} = 106.00$; Omuo: $F_{3, 84} = 166.46$ and Oye: $F_{3, 84} = 200.40$). The interactive effect between dose and exposure time on the mortality of bruchid obtained from each population was also significant ($p < 0.001$).

Impact of exposure time on the of susceptibility of diverse populations of *C. maculatus* from Ekiti State

The time (LT_{50}) needed to achieve 50% mortality in diverse populations of *C. maculatus* obtained from Ekiti State is shown in Table 5. Irrespective of the location, LT_{50} values decreased with increasing doses of *P. guineense*. However, at the highest and lowest experimental doses, bruchid obtained from Emure were the least susceptible (0.30g: 2.45 hrs; 0.05g: 1.29 hrs) while their counterpart from Ikere were the most susceptible (0.30g: 1.24 hrs; 0.05g: 0.62 hrs) to this plant material. At 0.25 and 0.30g, LT_{50} values of Ijero and Emure populations were not significantly different (p>0.05) from each other based on the overlapping fiducial limits. Likewise, at 0.05g, LT_{50} value of Emure (2.45 hrs) was not significantly higher (p>0.05) than that of their counterpart from Ijero (2.27 hrs) and Oye Ekiti (2.42 hrs) location. In summary, the order of susceptiblity of bruchid population to different doses of *P. guineense* from highest to lowest is shown in susceptibility pattern for each dosage below:

0.05g **-** Emure > Oye > Ijero > Ado > Omuo > Ikere

0.10g **-** Oye > Emure > Ijero > Ado > Omuo > Ikere

0.15g **-** Oye > Emure = Ijero > Ado > Omuo > Ikere

0.20g **-** Oye > Emure > Ijero > Ado > Omuo > Ikere

0.25g **-** Emure > Ijero > Ado > Omuo > Oye > Ikere

0.30g **-** Emure > Ijero > Ado > Oye = Omuo > Ikere

Table 1: Effect of *P. guineense* after 24 hour post treatment on the percentage mortality (Mean ± S.E.) of *C. maculatus* obtained from six geographical locations in Ekiti state

Dose (g/20g of cowpea)	Ado	Emure	Ijero	Ikere	Omuo	Oye
0.00	0.00 ± 0.00$^{a(A)}$	0.00 ± 0.00$^{a(A)}$	0.00 ± 0.00$^{a(A)}$	0.00 ± 0.00$^{a(A)}$	0.00 ± 0.00$^{a(A)}$	0.00 ± 0.00$^{a(A)}$
0.05	31.09±1.68$^{ab(A)}$	9.56± 1.13$^{ab(A)}$	21.00±1.11$^{b(A)}$	33.99±1.13$^{ab(A)}$	29.96±0.12$^{b(A)}$	17.00±0.11$^{ab(A)}$
0.10	33.52±0.95$^{b(AB)}$	12.57±0.02$^{ab(A)}$	25.00±0.07$^{b(AB)}$	47.23±0.230$^{bc(B)}$	35.04±1.13$^{b(AB)}$	18.00±1.12$^{ab(A)}$
0.15	34.43±1.01$^{b(ABC)}$	15.82±0.11$^{bc(A)}$	26.00±1.17$^{b(AB)}$	54.85±0.14$^{bc(C)}$	42.29±2.21$^{b(BC)}$	23.00±0.18$^{b(AB)}$
0.20	40.13±2.12$^{b(AB)}$	19.99±1.10$^{bc(A)}$	30.00±0.57$^{b(AB)}$	55.46±1.09$^{bc(B)}$	44.29±2.01$^{b(AB)}$	27.00±0.77$^{bc(A)}$
0.25	40.78±1.05$^{b(AB)}$	24.03±0.23$^{bc(A)}$	32.00±0.77$^{b(A)}$	61.15±2.23$^{bc(B)}$	48.54±0.87$^{b(AB)}$	47.00±1.13$^{cd(AB)}$
0.30	42.78±0.08$^{b(A)}$	28.30±0.55$^{c(A)}$	33.00±0.23$^{b(A)}$	81.25±1.19$^{c(B)}$	51.67±0.12$^{b(AB)}$	49.00±1.13$^{d(A)}$

Mean values followed by the same lower-case letter within a column are not significantly different at $p > 0.05$ by Tukey's test while means values followed by the same upper-case letter in bracket within a row are not significantly different at $p > 0.05$ by Tukey's test.

Table 2: Effect of *P. guineense* after 48 hour post treatment on the percentage mortality (Mean ± S.E.) of *C. maculatus* obtained from six geographical locations in Ekiti state

Dose (g/20g of cowpea)	Ado	Emure	Ijero	Ikere	Omuo	Oye
0.00	0.00 ± 0.00$^{a(A)}$	0.00 ± 0.00$^{a(A)}$	0.00 ± 0.00$^{a(A)}$	0.00 ± 0.00$^{a(A)}$	0.00 ± 0.00$^{a(A)}$	0.00 ± 0.00$^{a(A)}$
0.05	51.20±0.22$^{b(A)}$	34.52±0.23$^{b(A)}$	46.00±1.01$^{b(A)}$	83.48±0.24$^{b(B)}$	56.09±0.17$^{b(A)}$	37.15±1.13$^{b(A)}$
0.10	54.27±1.13$^{b(A)}$	44.09±0.87$^{b(A)}$	49.00±0.68$^{b(A)}$	88.99±1.37$^{b(B)}$	59.35±1.12$^{b(AB)}$	40.19±0.17$^{b(A)}$
0.15	58.40±1.19$^{b(AB)}$	68.92±1.00$^{c(B)}$	60.00±0.34$^{bc(AB)}$	91.97±0.11$^{b(C)}$	71.46±0.77$^{bc(B)}$	45.01±1.14$^{b(A)}$
0.20	62.76±0.18$^{b(A)}$	76.28±0.09$^{c(A)}$	71.00±0.23$^{c(A)}$	94.35±0.18$^{b(B)}$	77.05±1.13$^{bc(AB)}$	63.89±1.37$^{c(A)}$
0.25	66.49±1.10$^{b(A)}$	77.37±0.04$^{c(ABC)}$	74.00±0.73$^{c(AB)}$	95.83±1.00$^{b(C)}$	78.00±0.92$^{bc(ABC)}$	90.47±1.15$^{d(BC)}$
0.30	68.30±2.11$^{b(A)}$	82.03±1.12$^{c(AB)}$	78.00±0.27$^{c(AB)}$	97.92±0.19$^{b(C)}$	82.29±1.10$^{c(AB)}$	91.41±1.37$^{d(BC)}$

Mean values followed by the same lower-case letter within a column are not significantly different at $p > 0.05$ by Tukey's test while means values followed by the same upper-case letter in bracket within a row are not significantly different at $p > 0.05$ by Tukey's test.

Table 3: Effect of *P. guineense* after 72 hour post treatment on the percentage mortality (Mean ± S.E.) of *C. maculatus* obtained from six geographical locations in Ekiti state

Dose (g/20g of cowpea)	Ado	Emure	Ijero	Ikere	Omuo	Oye
0.00	0.00 ± 0.00$^{a(A)}$	0.00 ± 0.00$^{a(A)}$	0.00 ± 0.00$^{a(A)}$	0.00 ± 0.00$^{a(A)}$	0.00 ± 0.00$^{a(A)}$	0.00 ± 0.00$^{a(A)}$
0.05	70.30±0.36$^{b(AB)}$	59.64±0.23$^{b(A)}$	58.00±0.37$^{b(A)}$	89.91±1.98$^{b(B)}$	70.38±1.18$^{b(AB)}$	60.55±1.18$^{b(A)}$
0.10	72.22±1.19$^{bc(AB)}$	73.88±2.13$^{bc(AB)}$	65.00±1.18$^{bc(A)}$	95.53±2.13$^{bc(B)}$	72.60±0.91$^{b(AB)}$	63.91±0.23$^{b(A)}$
0.15	77.20±0.38$^{bc(AB)}$	82.63±1.10$^{cd(AB)}$	76.00±0.78$^{bcd(AB)}$	98.81±0.17$^{c(B)}$	86.94±1.17$^{c(AB)}$	66.50±2.35$^{b(A)}$
0.20	84.93±0.78$^{bc(AB)}$	95.34±0.10$^{cd(BC)}$	83.00±1.18$^{cde(AB)}$	98.81±0.17$^{c(C)}$	92.33±0.06$^{cd(ABC)}$	82.88±0.23$^{bc(A)}$
0.25	88.47±1.19$^{bc(A)}$	100.00±0.00$^{d(B)}$	90.00±0.03$^{de(A)}$	100.00±0.00$^{c(B)}$	94.46±0.02$^{cd(AB)}$	99.00±1.00$^{c(B)}$
0.30	90.07±0.17$^{c(A)}$	100.00±0.00$^{d(A)}$	95.00±1.91$^{e(A)}$	100.00±0.00$^{c(A)}$	100.00±0.00$^{d(A)}$	100.00±0.00$^{c(A)}$

Mean values followed by the same lower-case letter within a column are not significantly different at $p > 0.05$ by Tukey's test while means values followed by the same upper-case letter in bracket within a row are not significantly different at $p > 0.05$ by Tukey's test.

Table 4: Effect of *P. guineense* after 96 hour post treatment on the percentage mortality (Mean ± S.E.) of *C. maculatus* obtained from six geographical locations in Ekiti state

Dose (g/20g of cowpea)	Ado	Emure	Ijero	Ikere	Omuo	Oye
0.00	$0.00 \pm 0.00^{a(A)}$	$0.00 \pm 0.00^{a(A)}$	$0.00 \pm 0.00^{a(A)}$	$0.00 \pm 0.00^{a(A)}$	$0.00 \pm 0.00^{a(A)}$	$0.00 \pm 0.00^{a(A)}$
0.05	$76.95\pm0.13^{b(A)}$	$82.89\pm0.18^{b(A)}$	$73.00\pm1.12^{b(A)}$	$97.67\pm0.23^{b(A)}$	$75.77\pm1.19^{b(A)}$	$76.87\pm0.01^{b(A)}$
0.10	$78.13\pm1.17^{b(A)}$	$92.34\pm0.02^{bc(C)}$	$79.00\pm0.89^{bc(AB)}$	$100.00\pm0.00^{b(C)}$	$78.09\pm0.32^{b(A)}$	$91.33\pm0.02^{c(BC)}$
0.15	$87.55\pm0.67^{bc(A)}$	$93.24\pm1.18^{bc(A)}$	$85.71\pm0.77^{bcd(A)}$	$100.00\pm0.00^{b(A)}$	$97.73\pm1.10^{c(A)}$	$93.66\pm0.13^{c(A)}$
0.20	$91.36\pm1.18^{bc(A)}$	$100.00\pm0.00^{c(B)}$	$93.00\pm0.79^{cd(A)}$	$100.00\pm0.00^{b(B)}$	$100.00\pm0.00^{c(B)}$	$95.64\pm0.14^{c(AB)}$
0.25	$99.00\pm1.00^{c(A)}$	$100.00\pm0.00^{c(A)}$	$100.00 \pm0.00^{d(A)}$	$100.00 \pm0.00^{b(A)}$	$100.00 \pm0.00^{c(A)}$	$100.00 \pm0.00^{c(A)}$
0.30	$100.00\pm0.00^{c(A)}$	$100.00\pm0.00^{c(A)}$	$100.00\pm0.00^{d(A)}$	$100.00\pm0.00^{b(A)}$	$100.00\pm0.00^{c(A)}$	$100.00\pm0.00^{c(A)}$

Mean values followed by the same lower-case letter within a column are not significantly different at $p > 0.05$ by Tukey's test while means values followed by the same upper-case letter in bracket within a row are not significantly different at $p > 0.05$ by Tukey's test.

Table 5: Lethal time (LT_{50}) of different doses of *Piper guineense* powder required for diverse populations of *Callosobruchus maculatus* from Ekiti state.

Dose (g/20g of cowpea)	LT_{50} (Hours) of *C. maculatus* populations					
	Ado	Emure	Ijero	Ikere	Omuo	Oye
0.05	1.77	2.45	2.27	1.24	1.73	2.42
	(1.49-2.05)	(2.23-2.66)	(1.98-2.56)	(1.09-1.38)	(1.45-2.01)	(2.16-2.69)
0.10	1.64	2.03	1.98	1.04	1.53	2.09
	(1.36-1.92)	(1.86-2.20)	(1.72-2.24)	(0.90-1.17)	(1.24-1.81)	(0.67-3.51)
0.15	1.51	1.67	1.67	0.94	1.21	1.92
	(1.28-1.72)	(1.51-1.82)	(1.46-1.87)	(0.81-1.06)	(1.02-1.40)	(1.54-2.29)
0.20	1.31	1.45	1.42	0.93	1.14	1.53
	(1.10-1.51)	(1.33-1.57)	(1.24-1.59)	(0.80-1.05)	(0.46-2.36)	(1.36-1.69)
0.25	1.27	1.36	1.34	0.87	1.08	1.04
	(0.09-2.45)	(0.87-1.85)	(0.79-1.89)	(0.75-0.99)	(0.32-1.84)	0.92-1.15
0.30	1.23	1.29	1.28	0.62	1.02	1.02
	(0.00-2.46)	(0.91-1.67)	(1.15-1.40)	(0.37-0.87)	(0.00-2.04)	(0.91-1.13)

Values in parenthesis represent 95% confidence interval.

DISCUSSION

The effect of location on the susceptibility of diverse populations of *C. maculatus* obtained from six different towns across Ekiti state was investigated in this study. Consistent high bruchid mortality observed at the highest experimental doses and exposure time confirms the entomotoxic nature of this plant material to *C. maculatus* populations. This is in agreement with various findings reported by other studies on the insecticidal efficacy of *P. guineense* against *C. maculatus* (Ofuya *et al.*, 2010; Idoko and Adesina, 2012; Oyeniyi *et al*., 2015b). The high insecticidal effect of this plant material has been linked to a mixture of amides which enables the plant to employ analogue synergism defence strategy which augments its toxicity and renders it difficult for the insects to adapt and become resistant (Berenbaum and Zangerl, 1996; Feng and Isman, 1995). The powder of this plant material has been reported to cause physical abrasion of the cuticle leading to staggering, loss of direction (hallucination effect), motionlessness and finally death of exposed bruchid (Ogunwolu *et al*., 1998; Wang and Horng, 2004).

However, despite high mortality observed at the highest experimental doses, the pattern of susceptibility as revealed by LT_{50} values per dose of *P. guineense* shows that Ikere population of *C. maculatus* was the most susceptible irrespective of the dose while their counterpart from Oye and Emure were respectively the most tolerant at lower and higher doses. Consistent high susceptibility of bruchid population from Ikere may be linked to poor hygiene level in the town. Entomophagus bacteria and fungi are usually produced in grains that are poorly handled and stored in poor condition. For instance, entomoghagus fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* has been implicated to exhibit a limited amount of efficacy against *C. maculatus* on cowpea seeds poorly stored (Lopex-Meza and Ibarra, 1996; Vilas-Boas *et al*., 1996). The high mortality observed in bruchid population obtained from Ikere may therefore be linked to possible infection of bruchid sample from this town due to poor hygiene level. This might have reduced their innate ability to tolerate this plant material and thus, high bruchid mortality.

High tolerance of bruchid from Oye and Emure at lower and higher doses respectively may be linked to strategic position of both towns in Ekiti state. Oye and Emure were both located in the extreme Northern and Southern part of the state. Oye for example link Ekiti state to neighbouring Kwara state while Emure link the state to Ondo state. Both towns also play host to several markets usually patronized daily and/or weekly. Farm produce like banana and gaari are known to be very cheap in Ogbontoro market in Emure and this may have increased the influx of traders bringing their farm produce and products from neighbouring towns and states. The closeness of the state capital, Ado to Emure may have further lead to massive influx of traders in and out of this town. Due to the significant role *P. guineense* parts play as soup spices and in most herbal preparations, more seeds of this plant might have therefore being on sale in this town. Knowingly or unknowingly, bruchid population from this town might have come in contact with seeds of this plant material since diverse products are mostly stored together by most residence. Hence, high tolerance observed in bruchid population from Emure at the highest experimental doses might be linked to possible interaction between bruchid sample obtained from this town and the plant material. This might have increased their ability to tolerate the plant material more than their counterparts from other towns even at higher doses.

Also, the pattern of susceptibility to this plant material as revealed by LT_{50} values shows that from a dose of 0.05g to 0.20g, Ijero and Ado occupied the 3rd and 4th position which later changed to 2nd and 3rd position respectively at higher doses. The closeness of Ijero to Oye and Ado to Emure town may be responsible for the high tolerance observed in these two towns as the dosage of *P. guineense* increased. Higher duration needed in achieving 50% mortality in bruchid from both towns at higher doses further revealed that *C. maculatus* from both location became more tolerant of *P. guineense* as the doses increased.

Generally, the variation observed in the susceptibility of diverse populations of bruchid from Ekiti state to this plant material may be linked to cultural differences. Individuals from different locations usually differ in the way they think and handle food materials due to differences in culture and tradition. For bruchid on cowpea seeds to survive; they have to adapt to changes in their micro and macro environment. Consciously or unconsciously, new populations of insects may have being developed from time to time due to varying conditions in their immediate environment. Various conditions in their immediate environment might have therefore led to increase or decrease in the innate ability of diverse populations of *C. maculatus* from Ekiti state to tolerate powder of this plant material. This could thus be responsible for varying susceptibility observed in *C. maculatus* populations to this plant material. Similar observation has been reported by other scientists for some field to store pest using synthetic insecticides (Fragoso *et al*., 2002; Pereira *et al*., 2006; Odeyemi *et al*., 2010; Oyeniyi *et al*., 2015a).

In conclusion, botanicals could play a key role in ensuring proper management of bruchid on cowpea seeds to minimize loss by poor local farmers. Detailed knowledge is however needed to prevent possible future resistance which may occur overtime with the use of plant materials especially in large scale storage. Having a good knowledge on various factors that could possibly influence the efficacy of most botanicals is therefore needed to ensure their prospective usage as alternate insecticide to most synthetic insecticides.

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