

Resistance of *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) Populations in Nigeria to Dichlorvos

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Abstract

The resistance status of Nigeria populations of *Callosobruchus maculatus* (Fab.) to dichlorvos (2,3 – Dichlorovinyl dimethyl phosphate {DDVP}) was investigated in the present study. Bruchids were obtained from five different locations spread across three South-Western states of Nigeria. These include Akure (Ondo state), Ikare-Akoko (Ondo state), Ikere-Ekiti (Ekiti state), Ijan-Ekiti (Ekiti state) and Ibadan (Oyo state). A susceptible laboratory culture of *C. maculatus* served as the reference population. Bruchid populations were exposed to filter papers impregnated with DDVP (concentration range: 0.00001% - 0.01%) and the mortality was assessed after 3, 6, 24 and 48 hours post-treatment. Bruchid mortality varied across locations, DDVP concentration and exposure time. Bruchids obtained from Ibadan required the highest concentration (LC₉₅: 185.418 mg ml⁻¹) of DDVP, hence they have the highest resistance factor (RF) (RF₉₅: 1483.30); while their counterparts from Ijan-Ekiti required the lowest concentration of DDVP (LC₉₅: 0.242 mg ml⁻¹) with the lowest RF (RF₉₅: 1.94). The present study reveals diverse levels of resistance to DDVP in Nigerian populations of *C. maculatus*. Hence, there is a need for resistance management strategies on the use of DDVP and other organophosphate insecticide in its class across various Nigerian states to minimize cost and health risk implications that could arise from insecticide resistance.

Keywords: : Resistance, DDVP, Populations, *Callosobruchus maculatus*, Mortality.

1. Introduction

In spite of the widespread public concern about most synthetic insecticides on human health and environment, they are still heavily used and considered as the most effective method of controlling stored product pests in most nations, particularly for a large scale storage (Isman, 2000; Gbaye and Holloway, 2011). In Nigeria, for instance, the chemical control method is the most commonly used for pest management (Chedi and Aliyu, 2010). Even though the research on the use of botanical pesticides has gained prominence over the years, myriad of problems, such as relatively slow action, variable efficacy, instability in the environment, disagreeable odour, poor water solubility and inconsistent availability among others have trivialized the use of botanical pesticides against the newest generations of synthetic insecticides (Moretti et al., 2002; Isman and Grieneisen, 2014).

Synthetic insecticides, such as malathion, aluminium phosphide, pirimiphos-methyl, dichlorvos (dichlorovinyl dimethyl phosphate- DDVP), deltamethrin, cypermethrin and carbaryl, among others, are being used for controlling stored product pest either as fumigants or contact

insecticides (Desmarchelier, 1994; Zettler et al., 1997; Gbaye et al., 2012; Perveen and Khan, 2014). Although some of these chemicals have been banned in developed countries, some of them, especially dichlorvos (DDVP), are still being used to control households and stored products insects in some developing countries, including Nigeria (Chedi and Aliyu, 2010). DDVP is an organophosphate which has exhibited high efficacy against storage insect pests, both as contact and stomach poison (Rahman, 1990; Lotti, 2001; Booth et al., 2007).

Nigeria, being the largest producer and consumer of cowpea in the world as it accounts for 61% production in Africa and 58% worldwide, usually record huge post-harvest losses due to the debilitating effect of *Callosobruchus maculatus* on cowpea seeds (Singh and Ntare, 1985; IITA, 2010). Most cowpea merchants in Nigeria rely on the use of chemicals, such as DDVP to control this insect pest. Due to the high level of illiteracy among local farmers and post-harvest handlers of cowpea in Nigeria, pesticides are indiscriminately applied for insect pest control. Oyeniya et al. (2015a) opined that mismanagement of any form of insecticide can lead to resistance and loss of efficacy overtime. Insecticidal resistance, due to the failure of most chemicals to control insect pest, has also been implicated in the loss of food

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worth several billions of dollars globally (Elzen and Hardeen, 2003). Singh and Ntare (1985) reported that a 5% annual production loss to *C. maculatus* in Nigeria alone would cost about \$100 million. Therefore, the knowledge of insecticidal resistance status is needed to reduce the huge post-harvest losses associated with cowpea production and utilization in Nigeria.

The effect of vegetation on the resistance of cowpea Bruchids in Nigeria to pirimiphos-methyl has been investigated (Odeyemi et al., 2006). However, investigating the possible resistance of *C. maculatus* to DDVP in Nigeria has not been reported. Hence, the present study assessed the resistance status of *C. maculatus* populations in Nigeria to DDVP (2,3 – Dichlorovinyl dimethyl phosphate).

2. Materials and Methods

2.1. Insect Collection and Culture

The present study was carried out in the Biology Laboratory, Federal University of Technology Akure, Ondo state, Nigeria. *Callosobruchus maculatus* was obtained from infested cowpea seeds sourced from stores in five different locations across three states in Nigeria. These include: Akure (Ondo state), Ikare-Akoko (Ondo state), Ikere-Ekiti (Ekiti state), Ijan-Ekiti (Ekiti state) and Ibadan (Oyo state). Clean cowpea seeds (Sokoto white cultivar) were disinfested in the freezer at -18°C for two weeks, and prior to use, they were allowed to equilibrate in the laboratory for three days at ambient temperature and humidity ($28\pm 2^{\circ}\text{C}$ and $88\pm 5\%$) to prevent mouldiness. Bruchid samples from each location were introduced into 2.5 litres transparent plastic containers containing 200g of disinfested cowpea seeds and reared to F_1 generation. A susceptible laboratory culture of *C. maculatus* was obtained from Research Laboratory, Biology Department, Federal University of Technology, Akure, Ondo State, Nigeria. The laboratory culture served as the reference population and it was not exposed to synthetic insecticides or botanicals.

2.2. Preparation of the Chemical

2, 3-Dichlorovinyl dimethyl phosphate, DDVP, (Sniper[®] 1000EC) used for the experiment was obtained from an Agrochemical store in Ibadan, Oyo state, Nigeria. Various concentrations of the insecticide (DDVP) were prepared by dilution with acetone. The concentrations used were 0.00001%, 0.0001%, 0.0005%, 0.001%, 0.005% and 0.01%, while acetone only (0.0%) served as the control treatment.

2.3. Experimental Procedures

Impregnated filter paper technique, described in FAO method 15 (Anonymous, 1974) and modified for bruchids by Tyler and Evans (1981), was used to evaluate the resistance of *C. maculatus* to DDVP. Whatman's No.1 filter papers (110mm diameter) were treated with the varying concentrations of DDVP listed above. 0.5ml of a

concentration was applied to a filter paper with the aid of 1-ml syringe and the paper was allowed to air-dry for acetone to evaporate. Twenty unsexed adult *C. maculatus* were released onto each treated filter paper and covered with Petri-dish. Each treatment was replicated three times. Bruchids mated and laid eggs soon after adult emergence, hence the mortality was observed after 3, 6, 24 and 48 hours post-treatment. Bruchids were confirmed dead if there was no response when their abdomen is gently prodded with a needle. This procedure was done separately for all the Bruchid populations sampled and the various concentrations of the insecticides.

2.4. Statistical Analysis

Abbott formula (1925) was used to correct all data on adult mortality counts using control mortality. The data were subjected to analysis of variance (ANOVA) at $p < 0.05$ and the treatment means were separated using Tukey's Test. Data on adult mortality were subjected to probit analysis to determine the concentration of DDVP required to achieve 75% (LC_{75}) and 95% (LC_{95}) mortality in each sampled population (Finney, 1971). Data analysis was performed with Statistical Package for

$$\text{Resistance Factor (RF)} = \frac{\text{Lethal concentration of each population}}{\text{Lethal concentration of laboratory population}}$$

Social Sciences (SPSS) 17.0 software.

The resistance factor of each population was calculated from the lethal concentrations using the expression below:

(Modified from Oyeniyi et al., 2015a)

3. Results and Discussion

3.1. Response of *C. maculatus* Populations to DDVP

Tables 1-6 show the response of *C. maculatus* populations obtained from different locations to different concentrations of DDVP. Bruchid response varied with different concentrations and exposure times. There was a significant effect of DDVP concentration on the mortality of *C. maculatus* after a 3-hour exposure in all the populations (Akure: $F_{6,14} = 11.600$, $P < 0.0001$; Ikare-Akoko: $F_{6,14} = 17.694$, $P < 0.0001$; Ikere-Ekiti: $F_{6,14} = 7.833$, $P = 0.001$; Ijan-Ekiti: $F_{6,14} = 5.333$, $P = 0.005$; Ibadan: $F_{6,14} = 3.804$, $P = 0.018$), except for laboratory population ($F_{6,14} = 1.373$; $P = 0.291$). Similarly, at 6, 24 and 48 h post-treatment, significant effects ($P < 0.0001$) of DDVP concentration were observed in the bruchids. However, the highest effect was observed at 48 h post-treatment when compared to those exposed at other durations. After 48 hours of exposure, bruchids, obtained from all the locations, showed complete mortality (100%) at 0.005% and 0.01% concentrations, except for bruchids from Ibadan which had 100% mortality only at the highest experimental concentration (0.01%). For the laboratory population, complete mortality was observed at 0.001%, 0.005% and 0.01%.

Table 1. Effect of DDVP on the mortality (% mean \pm S.E.) of *C. maculatus* population from Akure

Concentration %	Duration (Hours)			
	3	6	24	48
0.0	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)}
0.00001	0.00 \pm 0.00 ^{a(a)}	0.00 \pm 0.00 ^{a(a)}	21.65 \pm 1.65 ^{b(b)}	36.65 \pm 4.40 ^{b(c)}
0.0001	0.00 \pm 0.00 ^{a(a)}	0.00 \pm 0.00 ^{a(a)}	30.00 \pm 5.75 ^{b(b)}	78.13 \pm 5.75 ^{b(c)}
0.0005	5.00 \pm 2.90 ^{b(a)}	18.35 \pm 6.00 ^{b(b)}	45.00 \pm 5.75 ^{b(c)}	87.55 \pm 5.75 ^{c(d)}
0.001	7.00 \pm 2.98 ^{ab(a)}	20.00 \pm 2.90 ^{b(b)}	65.00 \pm 5.75 ^{cd(c)}	91.36 \pm 2.90 ^{cd(d)}
0.005	10.00 \pm 2.98 ^{b(a)}	30.00 \pm 2.90 ^{b(b)}	73.35 \pm 12.00 ^{cd(c)}	100.00 \pm 0.00 ^{cd(d)}
0.01	18.35 \pm 1.65 ^{c(a)}	35.00 \pm 2.90 ^{b(b)}	76.65 \pm 6.00 ^{c(c)}	100.00 \pm 0.00 ^{cd(d)}

Mean values followed by the same letter(s) are not significantly different ($P>0.05$) by Tukey's Test. Letters immediately following the means are for vertical comparison while letters in parenthesis are for horizontal comparison.

Table 2. Effect of DDVP on the mortality (% mean \pm S.E.) of *C. maculatus* population from Ikare Akoko

Concentration %	Duration (Hours)			
	3	6	24	48
0.0	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)}
0.00001	0.00 \pm 0.00 ^{a(a)}	0.00 \pm 0.00 ^{a(a)}	25.00 \pm 2.90 ^{b(b)}	45.00 \pm 1.91 ^{b(c)}
0.0001	1.65 \pm 1.65 ^{a(a)}	3.35 \pm 1.65 ^{a(a)}	38.35 \pm 3.25 ^{c(b)}	51.65 \pm 4.05 ^{b(c)}
0.0005	3.35 \pm 1.65 ^{a(a)}	10.00 \pm 5.00 ^{a(a)}	40.00 \pm 5.00 ^{c(b)}	56.65 \pm 8.23 ^{b(c)}
0.001	13.35 \pm 3.35 ^{b(a)}	26.65 \pm 4.40 ^{b(b)}	51.65 \pm 4.40 ^{cd(c)}	96.65 \pm 0.00 ^{cd(d)}
0.005	20.00 \pm 2.90 ^{bc(a)}	50.00 \pm 5.75 ^{b(b)}	70.00 \pm 2.90 ^{cd(c)}	100.00 \pm 1.91 ^{cd(d)}
0.01	26.00 \pm 2.90 ^{c(a)}	55.00 \pm 5.75 ^{b(b)}	71.65 \pm 7.25 ^{cd(c)}	100.00 \pm 0.00 ^{cd(d)}

Mean values followed by the same letter(s) are not significantly different ($P>0.05$) by Tukey's Test. Letters immediately following the means are for vertical comparison while letters in parenthesis are for horizontal comparison.

Table 3. Effect of DDVP on the mortality (% mean \pm S.E.) of *C. maculatus* population from Ikere Ekiti

Concentration %	Duration (Hours)			
	3	6	24	48
0.0	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)}
0.00001	0.00 \pm 0.00 ^{a(a)}	0.00 \pm 0.00 ^{a(a)}	13.35 \pm 1.65 ^{b(b)}	45.00 \pm 7.25 ^{b(c)}
0.0001	0.00 \pm 0.00 ^{a(a)}	0.00 \pm 0.00 ^{a(a)}	20.00 \pm 5.75 ^{b(b)}	46.67 \pm 7.25 ^{b(c)}
0.0005	1.65 \pm 1.65 ^{a(a)}	13.35 \pm 3.35 ^{b(b)}	43.35 \pm 6.00 ^{c(c)}	91.65 \pm 1.65 ^{cd(d)}
0.001	5.00 \pm 2.90 ^{a(a)}	20.00 \pm 2.90 ^{bc(b)}	50.00 \pm 5.75 ^{cd(c)}	91.65 \pm 1.65 ^{cd(d)}
0.005	10.00 \pm 2.90 ^{ab(a)}	23.00 \pm 7.65 ^{bc(b)}	71.65 \pm 4.40 ^{cd(c)}	100.00 \pm 0.00 ^{cd(d)}
0.01	11.65 \pm 1.65 ^{ab(a)}	30.00 \pm 5.75 ^{b(b)}	73.65 \pm 4.40 ^{cd(c)}	100.00 \pm 0.00 ^{cd(d)}

Mean values followed by the same letter(s) are not significantly different ($P>0.05$) by Tukey's Test. Letters immediately following the means are for vertical comparison while letters in parenthesis are for horizontal comparison.

Table 4. Effect of DDVP on the mortality (% mean \pm S.E.) of *C. maculatus* population from Ijan Ekiti

Concentration %	Duration (Hours)			
	3	6	24	48
0.0	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)}
0.00001	0.00 \pm 0.00 ^{a(a)}	0.00 \pm 0.00 ^{a(a)}	21.65 \pm 2.00 ^{b(b)}	48.35 \pm 4.40 ^{b(c)}
0.0001	0.00 \pm 0.00 ^{a(a)}	0.00 \pm 0.00 ^{a(a)}	25.00 \pm 3.75 ^{b(b)}	50.00 \pm 1.75 ^{b(c)}
0.0005	3.35 \pm 1.00 ^{a(a)}	11.65 \pm 2.10 ^{ab(a)}	50.00 \pm 2.75 ^{c(b)}	70.00 \pm 3.15 ^{cd(c)}
0.001	4.12 \pm 1.65 ^{a(a)}	13.65 \pm 1.31 ^{b(a)}	60.00 \pm 1.65 ^{cd(b)}	85.00 \pm 2.65 ^{cd(c)}
0.005	6.00 \pm 1.13 ^{a(a)}	15.00 \pm 2.90 ^{b(a)}	65.00 \pm 0.75 ^{cd(b)}	100.00 \pm 0.00 ^{cd(c)}
0.01	10.00 \pm 2.90 ^{ab(a)}	23.35 \pm 4.40 ^{bc(b)}	88.35 \pm 3.40 ^{cd(c)}	100.00 \pm 0.00 ^{cd(d)}

Mean values followed by the same letter(s) are not significantly different ($P>0.05$) by Tukey's Test. Letters immediately following the means are for vertical comparison while letters in parenthesis are for horizontal comparison.

Table 5. Effect of DDVP on the mortality (% mean \pm S.E.) of *C. maculatus* population from Ibadan

Concentration %	Duration (Hours)			
	3	6	24	48
0.0	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)}
0.00001	0.00 \pm 0.00 ^{a(a)}	1.65 \pm 0.01 ^{a(a)}	20.00 \pm 2.90 ^{b(b)}	46.65 \pm 2.80 ^{b(c)}
0.0001	1.65 \pm 0.65 ^{a(a)}	5.00 \pm 1.90 ^{a(a)}	23.35 \pm 1.65 ^{b(b)}	50.00 \pm 5.75 ^{b(c)}
0.0005	3.35 \pm 0.65 ^{a(a)}	10.00 \pm 0.20 ^{ab(a)}	38.35 \pm 0.40 ^{c(b)}	53.35 \pm 2.40 ^{b(c)}
0.001	5.35 \pm 0.65 ^{a(a)}	16.65 \pm 1.05 ^{bc(ab)}	48.35 \pm 0.13 ^{cd(c)}	95.00 \pm 1.15 ^{cd(d)}
0.005	11.65 \pm 0.20 ^{a(a)}	26.65 \pm 1.30 ^{cd(b)}	50.00 \pm 0.75 ^{cd(c)}	97.00 \pm 1.00 ^{cd(d)}
0.01	13.65 \pm 1.40 ^{ab(a)}	30.15 \pm 1.40 ^{db)}	55.00 \pm 0.13 ^{dc)}	100.00 \pm 0.00 ^{cd(d)}

Mean values followed by the same letter(s) are not significantly different ($P > 0.05$) by Tukey's Test. Letters immediately following the means are for vertical comparison while letters in parenthesis are for horizontal comparison.

Table 6. Effect of DDVP on the mortality (% mean \pm S.E.) of *C. maculatus* population from Laboratory culture

Concentration %	Duration (Hours)			
	3	6	24	48
0.0	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)}
0.00001	0.00 \pm 0.00 ^{a(a)}	6.65 \pm 0.65 ^{a(a)}	30.00 \pm 2.65 ^{b(b)}	60.00 \pm 6.00 ^{b(c)}
0.0001	1.65 \pm 1.65 ^{a(a)}	9.65 \pm 1.12 ^{ab(a)}	41.65 \pm 2.00 ^{bc(b)}	61.65 \pm 5.75 ^{b(c)}
0.0005	11.65 \pm 0.33 ^{ab(a)}	16.65 \pm 1.65 ^{b(a)}	55.00 \pm 0.25 ^{c(b)}	90.00 \pm 5.75 ^{c(c)}
0.001	13.65 \pm 1.33 ^{b(a)}	21.65 \pm 0.40 ^{bc(a)}	73.35 \pm 0.65 ^{d(b)}	100.00 \pm 0.00 ^{c(c)}
0.005	14.35 \pm 0.00 ^{bc(a)}	24.65 \pm 1.15 ^{bc(a)}	78.35 \pm 0.25 ^{d(b)}	100.00 \pm 0.00 ^{c(c)}
0.01	16.35 \pm 0.35 ^{bc(a)}	27.00 \pm 0.65 ^{c(ab)}	88.35 \pm 0.40 ^{c(c)}	100.00 \pm 0.00 ^{cd(d)}

Mean values followed by the same letter(s) are not significantly different ($P > 0.05$) by Tukey's Test. Letters immediately following the means are for vertical comparison while letters in parenthesis are for horizontal comparison.

3.2. Lethal Concentrations of DDVP and Resistance Factors of *C. maculatus* Populations

The concentrations of DDVP required for achieving 75% (LC_{75}) and 95% (LC_{95}) mortality in the various populations of *C. maculatus* as well as each population's corresponding resistance factors after 24 hours post-treatment are shown in Table 7. Bruchids, obtained from Ibadan, required the highest concentration (LC_{75} : 0.316 mg ml⁻¹; LC_{95} : 185.418 mg ml⁻¹) of DDVP while their counterpart from Ijan-Ekiti required the lowest concentration of DDVP (LC_{75} : 0.006 mg ml⁻¹; LC_{95} :

0.242 mg ml⁻¹). Similarly, lethal concentration (LC_{75} and LC_{95}) values of bruchid population obtained from Ibadan were significantly higher ($P < 0.05$) than those obtained from Akure, Ikere-Ekiti and laboratory culture, respectively, as inferred from their fiducial limit values. Of all the populations sampled, the highest Resistance Factor (RF) was observed in bruchids population obtained from Ibadan (RF_{75} :158.00; RF_{95} : 1483.30), while the lowest RF was observed in those obtained from Ijan-Ekiti (RF_{75} :3.00; RF_{95} : 1.94).

Table 7. Lethal concentrations (LC_{75} and LC_{95}) (mg ml⁻¹) of DDVP and resistant factor of *C. maculatus* populations at 24 hour post-treatment

Location	Slope (\pm S.E)	LC_{75}	RF_{75}	LC_{95}	RF_{95}
Akure	0.56 (\pm 0.06)	0.007 (0.004-0.016)	3.50	0.411 (0.128-2.256)	3.29
Ikare-Akoko	0.43 (\pm 0.05)	0.022 (0.009-0.085)	11.00	3.963 (0.644-76.546)	31.70
Ikere-Ekiti	0.65 (\pm 0.06)	0.010 (0.006-0.022)	5.00	0.328 (0.120-1.364)	2.62
Ijan-Ekiti	0.61 (\pm 0.06)	0.006 (0.002-0.126)	3.00	0.242 (0.025-214.093)	1.94
Ibadan	0.35 (\pm 0.06)	0.316 (0.066-5.329)	158.00	185.418 (9.308-48677.02)	1483.30
Laboratory	0.56 (\pm 0.06)	0.002 (0.001-0.004)	1.00	0.125 (0.045-0.543)	1.00

SE: Standard error; LC: Lethal concentration; RF: Resistance factor. Values in parenthesis represents 95% Fiducial limits.

4. Discussion

In the present study, the response of various Nigerian populations and laboratory culture of *C. maculatus* to DDVP was evaluated. The results obtained indicated that the mortality of bruchid samples from each location varied with different concentrations of insecticide and exposure time. Except for the Laboratory (reference) population, less than 50% mortality was observed in all the locations with the lowest experimental concentration even at the highest duration (48 hours). *C. maculatus* is known to mate shortly after emergence, with the majority of eggs laid within three days (Fox, 1993; Ofuya, 1995). Hence, bruchid populations, in the present study, would have mated and laid egg before being killed. Although DDVP is known to be active against immature stages of stored product insects within grains (Semple et al., 1992), eggs already laid by the adult bruchid usually lead to a loss in the aesthetic and the marketability value of the infested cowpea seeds (Swella and Mushobozy, 2007). This might be responsible for post-harvest losses usually incurred on stored cowpea seeds in Nigeria despite the use of synthetic insecticides in most cases (Singh and Ntare, 1985; Baidoo et al., 2010).

Insecticide resistance refers to the insecticide selected inheritable ability of insects' population to withstand the exposure to a dose of an insecticide that would kill the majority of a normal (susceptible) population of the same species (Buhler, 2013). Lethal concentrations and resistance factors of the sampled populations revealed that Ibadan population of *C. maculatus* showed the highest resistance to DDVP. High resistance of the bruchid sample from Ibadan may be linked to the strategic location of this city, being in Southern-Western part of Nigeria. Ibadan is the largest city in West Africa and the second largest in all Africa (Kumassah, 2009). It is also the third cheapest Nigerian city to live in and it contains a large human population (about 2.949 million as at 2011) (Ejiofor, 2014; NDP, 2014). There are several markets and storage facilities within and around the metropolis. Hence, more insecticides might have been used in the management of diverse stored product pests in most stores. For instance, in Nigeria, DDVP is one of the most common insecticides used directly on cowpea seeds before being bagged for storage. The repeated exposure of bruchids to this insecticide overtime might have led to their possible resistance to this insecticide. On the contrary, the low resistant factor of the bruchid population from Ijan-Ekiti to DDVP suggests that this insecticide might not have been over-used on bruchids sampled from this town which is the smallest town of all the locations sampled. Frago et al. (2002), Pereira et al. (2006) and Odeyemi et al. (2010) had earlier ascribed the variation in the resistance of insect from different locations to the greater use of insecticides and to the usage pattern in those locations.

The differences in the resistance factor between insect populations to a particular insecticide have been attributed to several factors. Such factors include: thickness of insect's exoskeleton, type of insecticide being used, the ability of the insect to metabolize a poison, concentration of insecticide used, time of exposure, the type of food

eaten by the insect, insect location and species, among other parameters (Gbaye et al., 2011; Buhler, 2013; Oyeniyi et al., 2015a; Oyeniyi et al., 2015b). Some of these factors might have contributed to the variations observed in the resistance factor of Bruchid populations sampled in the present study. Variations in the resistance status of Bruchid populations observed in the present study is in line with the various reports on the resistance of diverse insect pests to synthetic insecticides (Jermannaud, 1994; Perez et al., 2000; Pereira et al., 2006).

A concentration of DDVP higher than 0.01% (0.01 mg ml⁻¹ which is the maximum residue limit (MRL) permitted by EU) would be required for effectiveness within 24 hours. However, due to various adverse effects associated with pesticide usage as well as a recent ban imposed on Nigeria by European Union owing to high level of DDVP (0.03-4.60 mg kg⁻¹) in exported cowpea grains (Nigeria Punch of 30th July, 2015), increasing the concentration above 0.01% cannot be encouraged. Thus, to reduce the huge post-harvest losses, usually incurred due to inability of insecticides to ensure maximum protection of cowpea, there is a dire need to constantly monitor and manage the resistance to DDVP. In areas where resistance is observed, such as Ibadan, there is a need to discontinue the use of DDVP and replace it with other insecticides that have a different chemistry and mode of action. This is required while the search for sustainable alternative control measures to synthetic chemicals is on, especially under a large scale storage where botanical use is not realistic.

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