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# INTERACTIVE EFFECT OF COWPEA VARIETY, DOSE AND EXPOSURE TIME ON BRUCHID TOLERANCE TO BOTANICAL PESTICIDES

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## ABSTRACT

*Callosobruchus maculatus* has for years remained a serious menace in cowpea in Sub-Sahara Africa. The objective of this study was to investigate the effect of genotypic cowpea (*Vigna unguiculata* (L.) Walp) varieties, time and dose on *C. maculatus* exposed to powders of *Piper guineense* and *Eugenia aromatica*. Irrespective of duration and botanicals, bruchid reared on KDV showed the highest tolerance to both plant materials; while their counterparts from IAR48V were the most susceptible. Median lethal time ($LT_{50}$) also varied according to the plant materials; with the highest in KDV reared bruchid [*P. guineense*: KDV (18.31), IAR48V (9.27), IFBV (13.17); *E. aromatica*: KDV (76.01), IAR48V (5.59), IFBV (6.49)]. There was a significant impact of cowpea variety (V), exposure time (T) and dose (D) on the tolerance of *C. maculatus* to both plant materials. The effect of all two-way (VxT, VxD, DxT) and three way interactions (V×T×D) on the tolerance of *C. maculatus* to both plant materials was also significant. Varietal effect was more pronounced in bruchids exposed to *E. aromatica;* while exposure time was more pronounced in bruchids exposed to *P. guineense*.

*Key Words*: *Callosobruchus maculatus*, *Eugenia aromatica*, *Piper guineense*

## RÉSUMÉ

*Callosobruchus maculatus* a été pendant plusieurs années une menace pour la culture du niébé en Afrique au Sud du Sahara. L'objectif de cette étude est d'évaluer l'effet des génotypes de niébé (*Vigna unguiculata* (L.) Walp), le temps d'exposition et la dose de poudre de *Piper guineense* and *Eugenia aromatica* administrée sur *C. maculatus*. Indépendamment au temps d'exposition, les bruches élevées sur KDV se sont montrées les plus tolérantes aux poudres des deux plantes; tandis que leur homologues élevées sur IAR48V étaient les plus susceptibles. Le temps de demi-vie ($LT_{50}$) aussi varie selon la plante dont la poudre est administrée; avec le temps de demi-vie le plus élevé observé chez les bruches développées sur KDV [*P. guineense*: KDV (18.31), IAR48V (9.27), IFBV (13.17); *E. aromatica*: KDV (76.01), IAR48V (5.59), IFBV (6.49)]. L'étude a révélé une très grande influence sur les variétés de niébé (V), le temps d'exposition (T) et la dose (D) sur la tolérance de *C. maculatus* aux deux espèces végétales. L'effet de toutes les interactions de deux (VxT, VxD, DxT) ou des trois facteurs (V×T×D) sur la tolérance de *C. maculatus* aux deux espèces végétales était aussi significatif. L'effet de génotype était plus prononcé sur les bruches exposées à *E. aromatica;* tandis que l'effet de temps d'exposition était plus prononcé sur les bruches soumises à *P. guineense*.

*Mots Clés*: *Callosobruchus maculatus*, *Eugenia aromatica*, *Piper guineense*

## INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is a grain legume which plays a vital nutritional role globally, particularly in developing countries where it serves as an important source of protein, carbohydrate and vitamins. Nigeria is the largest cowpea producer in the world, followed by Niger (FAOSTAT, 2013).

Production of this crop faces enormous problems; notable among them is insect pest infestation. Post-harvest losses to storage insect pests limit cowpea production in sub-Saharan Africa, which otherwise accounts for about 70% of total world production (IITA, 2010). In Nigeria as much as 10% of the cowpea seeds may be damaged before it is stored (Yusuf, 2009). Bruchids, especially those belonging to genus *Callosobruchus* are a menace to this legume. This genus contains several cosmopolitan, tropical and subtropical pests of grain legumes, of which *Callosobruchus maculatus* (F.) is the most prominent. It accounts for over 90% of the damage done to stored cowpea seeds (Caswell, 1981).

Diverse measures have been used to control this insect pest. Notable among them is the introduction of cowpea varieties resistant to bruchids attack. Most cultivated cowpeas are quite susceptible to bruchid damage (Dobie, 1981; 1986) and due to the strategic significance of this crop in the world, research on its long-term genetic improvement is ongoing within national laboratories and institutions of higher education in several West African and Western countries of the world (Owolabi *et al*., 2012). Breeders aim at generating varieties with better nutritional composition, higher yield, early maturity, diseases resistance and resistance to insect pest attack (Singh and Singh, 1990; Ofuya, 2001). Consequently, genetically distinct varieties are being produced in different countries to increase resistance to bruchids. For instance, workers at the International Institute of Tropical Agriculture (IITA) in Nigeria have screened several cowpea germplasm, but with only TVu 2027 being resistant to bruchid attack (Singh, 1978).

The use of botanicals on subsistence level has also provided an alternative control measure for *C. maculatus,* especially in developing countries. These materials are known to be cheap and eco-friendly (Akinkurolere *et al*., 2006; Adebiyi and Tedela, 2012). Thus, the use of plant products could have a substantial role to play in increasing cowpea production (Singh, 2011). Despite high success reported on the efficacy of most plant materials in controlling bruchids, there is dearth of information on the potential impact of cowpea variety as well as its interactive effect with exposure time and dose on the tolerance of *C. maculatus* to the botanicals (Gbaye *et al*., 2011; Gbaye and Holloway, 2011). This study investigated the multifactorial effect of cowpea variety, exposure time and dose on the tolerance of *C. maculatus* to powders of *Piper guineense* seed and *Eugenia aromatica* flower buds.

## MATERIALS AND METHODS

The experiment was carried out during 2013 to 2014, at the Storage Entomology Research Laboratory of Biology Department, Federal University of Technology Akure, Nigeria. Dry seeds of *P. guineense* (African black pepper) and dry flower buds of *E. aromatica* (Cloves) were purchased from local herbs seller at Olufi market, Gbongan, Osun State in Nigeria. The plant materials were pulverised in the laboratory using NAKAI NJ-1731 electric blender. They were further sieved with a mesh size of 1 $mm^2$, before being stored in plastic containers with airtight lids. This procedure was carried out separately to avoid plant materials contaminating each other.

Ife Brown (designated: IFBV) and IAR48 (designated: IAR48V) cowpea varieties used for this study were obtained from National Seed Service (NSS), Ibadan, Nigeria; while the third variety, Kannanado (designated: KDV), was obtained from Akure, Nigeria. They were disinfested by freezing at -18 $^{o}$C for four weeks, and thereafter, allowed to equilibrate for three days at ambient temperature and humidity (28 $\pm$ 3 $^{o}$C and 75 $\pm$ 6% relative humidity) in the laboratory, prior to use, to prevent mould formation.

The starter culture of *C. maculatus* (Cameroon strain) used for the study was obtained from the Centre for Wildlife Assessment and Conservation of the University of Reading, Reading, UK. This strain has been maintained in the laboratory for over 300 generations, without exposure to synthetic insecticides or plant materials. This same strain was used for study with synthetic insecticide by Gbaye and Holloway (2011).

The bruchids were reared for two generations on each cowpea variety separately to eliminate maternally inherited dietary effects (Gbaye *et al*., 2011). The insects were cultured in 1.65 litre plastic containers. The containers were covered with perforated covers sealed with muslin cloth, to prevent insect escape and to facilitate air exchange. The insects were maintained on each cowpea variety, without exposure to either synthetic insecticides or plant materials at an ambient temperature (28 $\pm$ 3 ºC) and relative humidity (75 $\pm$ 6%).

All the varieties of cowpea seeds used in this study were observed to be relatively susceptible to *C. maculatus* attack. However, KDV seeds were the most susceptible to bruchids attack, with the fewest days required for adult emergence. Bruchids reared on KDV were also the largest in size from the three varieties.

Ten grammes of cowpea seeds were taken using a Metler beam weighing balance, and placed into each of twenty four 170 ml plastic containers (8.7 cm in diameter). The seeds were thoroughly mixed with 0.00 (control), 0.05, 0.10, 0.15, 0.20 or 0.25 g of *P. guineense* powder.

Each treatment was replicated four times. Twenty five 1-3 days old adult *C. maculatus* were introduced into each replicate container and covered with perforated covers sealed with muslin cloth. Insect response to this plant material was assessed after 12, 24 and 48 hours, post-treatment, using dead insects as indicators. Bruchids were confirmed dead when there was no response to abdomen gently prodding with a needle. The above procedure was carried out separately for bruchids that emerged from each experimental cowpea variety.

The procedure used for *P. guineense* powder was repeated with *E. aromatica* powder, but at lower doses of 0.00 (control), 0.04, 0.05, 0.06, 0.07 and 0.08 g per 10 g of cowpea, because of the higher toxicity of *E. aromatica* powder to bruchids (preliminary test).

Twenty cowpea seeds from each variety were randomly selected for measurement of length, width and thickness, using a micrometer screw guage (model - RQHS NORM 2002/95/EC). Other seed characteristics; namely texture, hilium colour and seed coat colour were assessed visually with the aid of hand lens.

Abbott (1925) formula was used to correct data on adult mortality counts using control mortality. The data were subjected to analysis of variance ($P<0.05$) and where significant difference existed, means were separated using Tukey's test. Data on adult mortality were also subjected to probit analysis, to determine the lethal time required by both plant materials to kill 50% ($LT_{50}$) of *C. maculatus* from each variety. General Linear Model (GLM) was used to determine the significant interaction of variety, exposure time and dose on the tolerance of *C. maculatus* to both plant materials. All analyses were carried out using Statistical Package for Social Sciences (SPSS) 17.0 Software Package.

## RESULTS

**Seed morphometrics and characteristics.** Table 1 shows seed morphometrics and characteristics of the cowpea varieties used for this study. KDV seeds were the longest, widest and thickest. IAR48V seeds were longer and wider than those of IFBV, but seeds of IFBV were thicker than those of IAR48V. The differences in length, width and thickness between the varieties were highly significant ($P<0.0001$).

**Effect of *P. guineense* powder.** Irrespective of the cowpea variety from which *C. maculatus* emerged, tolerance of bruchids to *P. guineense* decreased with increase in exposure time (Fig. 1). Bruchids reared on IAR48V, however, showed the lowest effect to exposure time ($P<0.0001$); while their counterpart reared on KDV showed the highest effect. Likewise, there was significant effect of dose (D) ($P<0.0001$) on the tolerance of *C. maculatus* to *P. guineense*. IFBV reared bruchids, however, showed the lowest response to dose effect; while the highest response was observed in KDV reared bruchids.

After 12 hours (Fig. 1A), regardless of the dose of *P. guineense*, the mortality values of bruchids reared on KDV were significantly lower ($P<0.05$) than those reared on IFBV and IAR48V; while

TABLE 1. Seed morphometrics and characteristics of three Nigerian cowpea varieties

| Variety | Seed morphometrics | | | Seed characteristics | | |
|---|---|---|---|---|---|---|
| | Length (mm) | Width (mm) | Thickness (mm) | Texture | Hilium colour | Coat colour |
| IFBV | 7.43±0.10[a] | 5.59±0.07[a] | 4.03±0.08[b] | Wrinkle | White | Light brown |
| IAR48V | 8.48±0.14[b] | 5.96±0.08[b] | 3.73±0.06[a] | Wrinkle | White | Deep brown |
| KDV | 9.83±0.20[c] | 7.39±0.11[c] | 6.09±0.10[c] | Wrinkle | White | White |

Each value is the mean±S.E of 20 cowpea seeds. Means within the same column followed by different letter are significantly different (P< 0.05) from each other according to Tukey's test

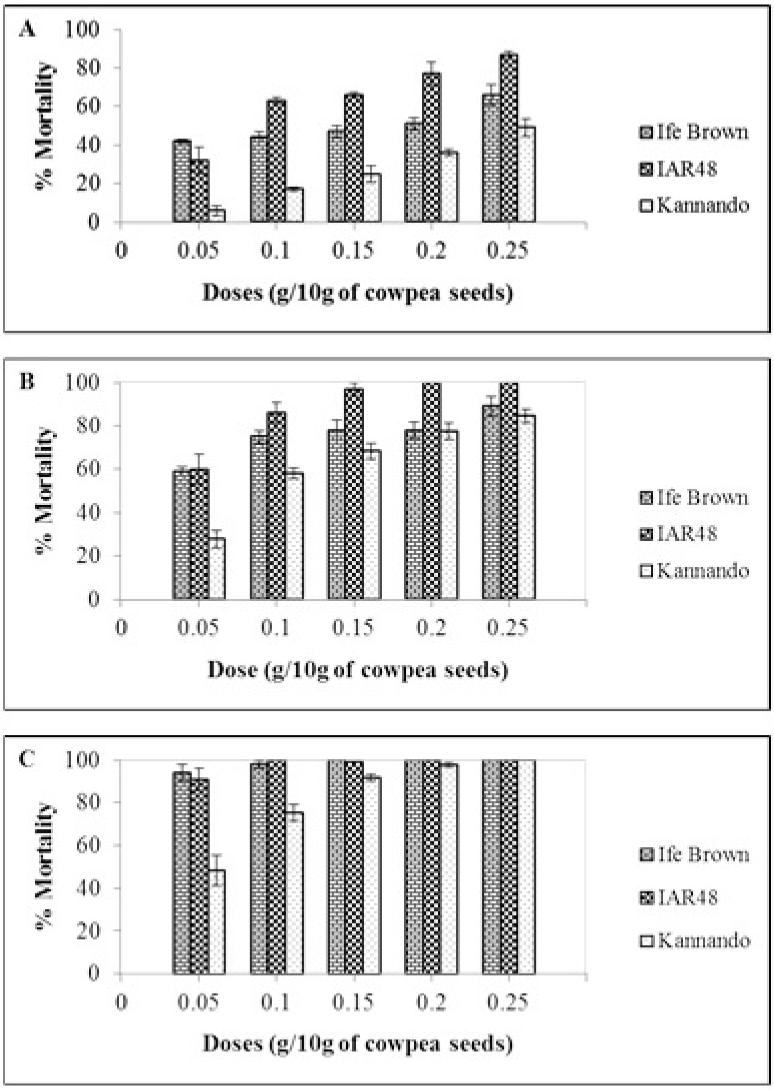


Figure 1. Varietal effect of cowpea on the tolerance of *C. maculatus* to *P. guineense* powder after (A) 12 hours, (B) 24 hours and (C) 48 hours.

those reared on IFBV were generally significantly lower (P<0.05) than those reared on IAR48V. After 24 hours (Fig. 1B), there was no significant difference (P>0.05) between the response of KDV and IFBV reared bruchids exposed to 0.20 and 0.25 g. Also, 100% mortality was observed in the IAR48V reared bruchids at 0.20 and 0.25 g after 24 hour exposure. After 48 hours, no significant difference (P>0.05) was observed between the mortality of bruchids reared on IFBV and IAR48V, irrespective of the *P. guineense* dosage.

**Effect of *E. aromatica* powder.** As earlier observed in bruchids exposed to *P. guineense*, regardless of the cowpea variety from which *C. maculatus* emerged, tolerance to *E. aromatica* powder decreased with increase in exposure time, with the highest tolerance shown by bruchids reared on KDV (highest mortality: 88.83% at 0.08 g after 48 hours) (Fig. 2). However, there was a significant effect of exposure time (P<0.0001) on the tolerance of bruchids to *E. aromatica,* with the lowest effect shown on IFBV-reared bruchids;

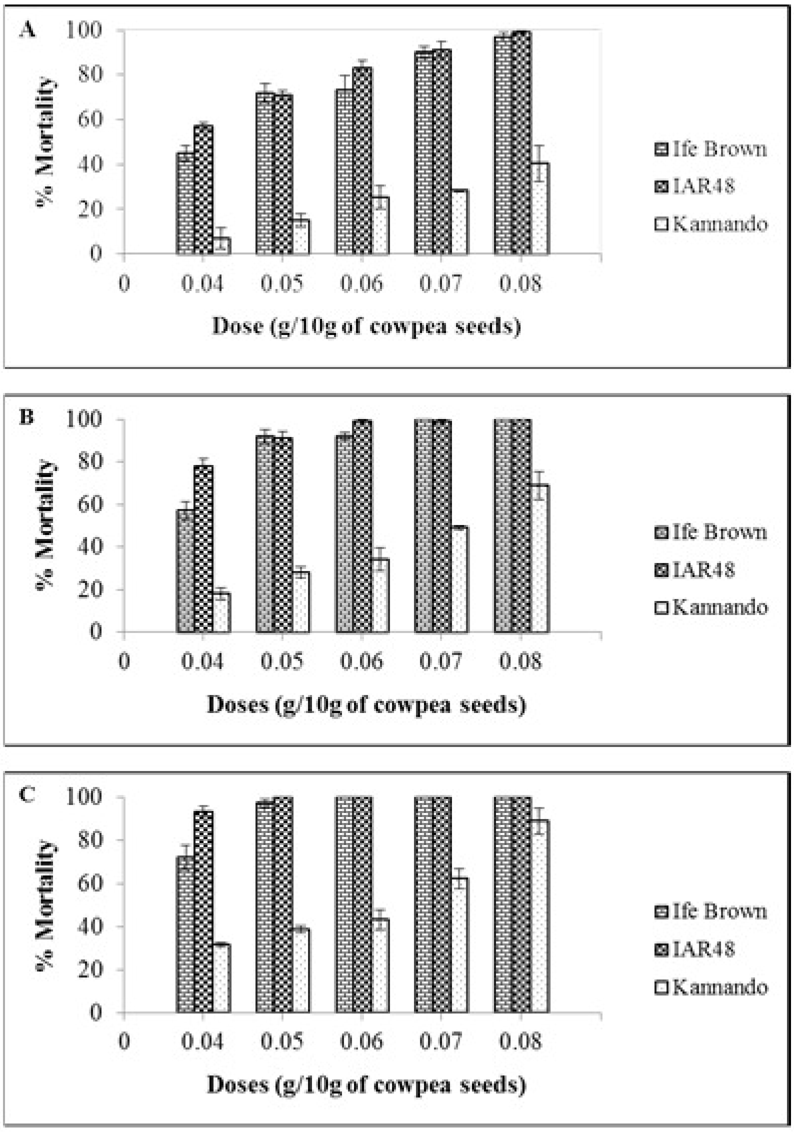


Figure 2. Varietal effect of cowpea on the tolerance of *C. maculatus* to *E. aromatica* powder after (A) 12 hours, (B) 24 hours and (C) 48 hours.

while the highest effect was observed in IAR48V-reared bruchids. The effect of dose was also highly significant. KDV-reared bruchids showed the lowest response to dose effect of *E. aromatica* and their counterparts reared on IAR48V showed the highest response to dose effect.

Irrespective of doses of *E. aromatica* powder, the mortality of bruchids reared on KDV was significantly lower ($P<0.05$) than those reared on IFBV and IAR48V, except at 0.08 g after 48 hours, where the mortality of KDV reared bruchids was not significantly different ($P>0.05$) from those reared on IFBV and IAR48V (Fig. 2). Similarly, there was no significant difference ($P>0.05$) between the mortality values of bruchids reared on IFBV and IAR48V after 12, 24 and 48 hours post treatment.

**Lethal time ($LT_{50}$) of plant powders.** Table 2. shows the lethal time ($LT_{50}$) of different doses of *P. guineense* and *E. aromatica* powder against *C. maculatus*. The highest $LT_{50}$ values were observed in KDV-reared bruchids, regardless of the botanicals and their doses. This was closely followed by bruchid lines reared on IFBV. However, the $LT_{50}$ values at a dose of 0.05 g of *P.*

TABLE 2. Lethal time ($LT_{50}$) of different doses of *P. guineense* and *E. aromatica* powder against *C. maculatus* from three cowpea varieties

| Doses of *P. guineense* | $LT_{50}$ (Hours) | | |
|---|---|---|---|
| | Ife Brown variety | IAR48 variety | Kannando variety |
| 0.05g | 15.89<br>(12.47-19.31) | 18.1<br>(13.64-22.38) | 47.5<br>(37.86-57.14) |
| 0.10g | 13.82<br>(11.69-15.95) | 9.86<br>(6.87-12.85) | 24.05<br>(20.98-27.12) |
| 0.15g | 13.17<br>(10.91-15.43) | 9.2<br>(4.65-13.75) | 18.31<br>(16.36-20.26) |
| 0.20g | 12.46<br>(10.12-14.80) | 8.49<br>(5.45-11.53) | 14.99<br>(13.54-16.44) |
| 0.25g | 9.27<br>(5.75-12.79) | 6.21<br>(4.21-8.21) | 12.46<br>(10.78-14.14) |
| ***E. aromatica*** | | | |
| 0.04g | 15.88<br>(8.74-23.02) | 10.06<br>(7.26-12.86) | 92.81<br>(78.92-106.7) |
| 0.05g | 7.25<br>(2.90-11.60) | 8.26<br>(5.53-10.99) | 78.4<br>(60.25-96.55) |
| 0.06g | 6.49<br>(2.88-10.10) | 5.59<br>(2.60-8.58) | 76.01<br>(40.22-111.8) |
| 0.07g | 5.44<br>(3.11-7.77) | 2.44<br>(0.00-4.90) | 27.85<br>(24.84-30.86) |
| 0.08g | 1.89<br>(0.00-3.79) | 0.29<br>(0.00-0.58) | 15.08<br>(9.18-20.98) |

Values in parenthesis represent 95% fiducial limits *guineense* (18.10 hours) and *E. aromatica* (8.26 hours) were slightly higher in insects reared on IAR48V, compared with those reared on IFBV. But generally, bruchids reared on IAR48V were the most susceptible to both botanicals with the lowest $LT_{50}$ values at each of the doses of both botanicals. Likewise, based on the overlapping fiducial limits at 0.20 and 0.25 g of *P. guineense*, $LT_{50}$ values for KDV-reared bruchids were not significantly higher ($P>0.05$) than those of IFBV reared bruchids.

**Interactive effect of variety, duration and dose.** The interactive effects of cowpea variety-by-dose and cowpea variety-by-duration on bruchid tolerance to *P. guineense* and *E. aromatica* are shown in Figures 3 and 4, respectively. General Linear Model (GLM) revealed a highly significant ($P<0.0001$) effect of cowpea variety on the tolerance of *C. maculatus* to *P. guineense* and *E. aromatica*. Likewise, the effects of exposure time and dose on the tolerance of *C. maculatus* to both botanicals were highly significant. Similarly, there were significant impacts of all interactions on mortality of bruchids exposed to both botanicals. Varietal effect was more pronounced in bruchids exposed to *E. aromatica* while exposure time was more pronounced in bruchids exposed to *P. guineense*.

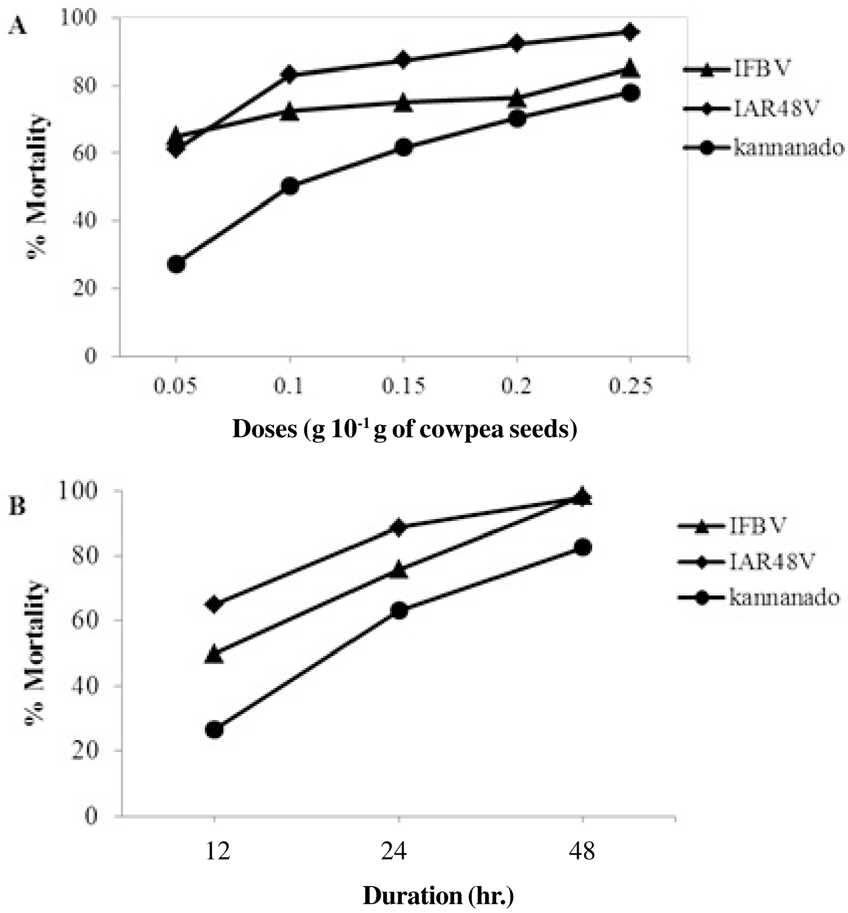


Figure 3. Interactive effect of (A) cowpea variety-by-dose (average across time) and (B) cowpea variety-by-duration (averaged across dose) on the tolerance of *C. maculatus* to *P. guineense* powder.

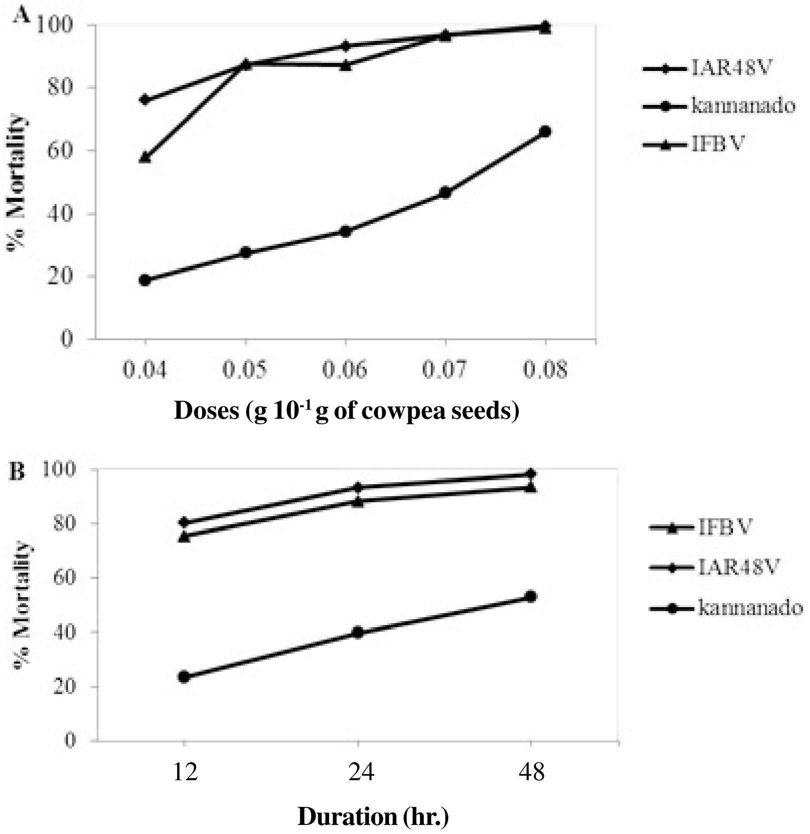


Figure 4. Interactive effect of (A) cowpea variety-by-dose (average across time) and (B) cowpea variety-by-duration (averaged across dose) on the tolerance of *C. maculatus* to *E. aromatica* powder.

## DISCUSSION

Irrespective of treatment, increasing dose in the form of powder of either of these plants added to whole cowpeas resulted in an increase in adult bruchids mortality. This demonstrates that the plants have protective properties against *C. maculatus,* thus corroborating previous findings by Gbewonto *et al.* (1993) for *P. guineense* and Ofuya *et al.* (2010) for *E. aromatica*. Many plants produce secondary compounds for protection against insect herbivores (Harborne, 1988). The most likely active compounds here are the amides pipercide and chavicine within *P. guineense* (Su, 1977; Miyakado *et al* 1979) and the volatiles eugenol, caryophylline and oleanol for *E. aromatica* (Huang *et al.,* 2002; Pungitore *et al*., 2005; Bhowmik *et al*., 2012).

The cowpea variety on which the bruchids were reared had a significant impact on tolerance to *P. guineense* and *E. aromatica*. The varieties used varied considerably in size and appearance. The fact that the cowpea variety on which the bruchids were reared influenced tolerance to the botanicals, indicates that the cowpeas varied in chemical constituents and, in turn, this influenced the physiology of the developing bruchids. Secondary compounds accumulate, in particular, in the seed coat, including tannins, flavonoids and phenolic acids (Lattanzio *et al.,* 1997; Egounlety and Aworh, 2003). It is known that the grain used for rearing influences insect physiology (Holloway and Mackness, 1988) by inducing different arrays of enzymes to varying extents (Gbaye *et al.*, 2012). Enzyme induction, particularly on toxic foodstuffs such as peas and beans, is energetically expensive and affects several life history characters (Holloway *et al.,* 1990; Povey and Holloway, 1992). One life history character that is affected is adult survival in the face of toxic xenobiotics, such as synthetic insecticides.

Gbaye and Holloway (2011) demonstrated that the cowpea variety on which *C. maculatus* is reared influences tolerance to malathion. Given this, a varietal effect on tolerance to natural insecticides is perhaps less surprising. What we do not know is whether energy into the detoxification of cowpea secondary compounds makes the insects more likely to tolerate botanical insecticides or whether a less toxic cowpea makes more energy available to deal with toxic xenobiotics.

Insects emerging from the KDV variety were most tolerant of *P. guineense* and *E. aromatica* and KDV is a white coated variety containing fewer toxins than brown coated varieties. Interestingly, Gbaye and Holloway (2011) also found that the white coated variety, NCRI-L25, produced adult *C. maculatus* more tolerant of malathion than the coloured cowpea seeds used.

The second explanation for the variation in tolerance is that different sized individuals (on average) emerge from the different cowpea varieties. The variety KDV is considerably larger than the other two varieties used (Table 1). Although body size was not a character measured in the present study, it is not inconceivable that a larger cowpea could result in larger bruchids. *Callosobruchus maculatus* distributes its eggs among cowpeas in an even manner (Messina and Mitchell, 1989), but thereafter, the larvae exhibit scramble competition for food (Mitchell, 1991). When food runs out, the insects pupate and emerge as small adults. It follows that a large cowpea seed will offer greater food reserves than a small cowpea, and the adult insects emerging are likely to be larger (Willmer *et al*., 2000). It is well known that large individuals are more tolerant of toxins (Holloway, 1986; Delorme *et al*. 1988; Buhler, 2013) and the bruchids emerging from the largest cowpea variety were indeed more tolerant of *P. guineense* and *E. aromatica*.

The degrees of interactions among cowpea variety, exposure time and dose have different impact on the susceptibility of *C. maculatus* to both botanicals (Figs. 3 and 4). While it was the effect of cowpea variety that was the prominent factor in bruchids that were exposed to *E. aromatica* (higher F value), exposure time was the most influential in insects exposed to *P. guineense*. This may be responsible for higher mortality values of IFBV and IAR48V reared bruchids exposed to *E. aromatica* powder after 24 and 48 hours, compared with KDV reared bruchids.

Research remains to be done to understand the factors influencing variation in tolerance in adult bruchids in relation to cowpea variety. This is important in a practical sense in that cheap and accessible ways of managing cowpea damage by bruchids are highly desirable. Readily available botanicals offer a suitable and safe alternative to synthetic compounds, but as with synthetic insecticides farmers need to be advised of a single and effective field dose. It is clear from this study that the cowpea variety being grown is likely to influence the field dose required to affect satisfactory control. Furthermore, as shown in Table 2, the $LT_{50}$ values sometimes span two or three days depending on dose. *Callosobruchus maculatus* adults in the absence of liquid food only live for about five days (Møller *et al.,* 1989). Before the insects succumb to the toxic effects of the botanicals, it is possible that they are still able to oviposit. Despite all these questions, the use of botanicals as a means of controlling bruchid damage to cowpea shows considerable promise.

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