

INSECTICIDAL EFFECTS OF *DENNETTIA TRIPETALA* (BAK.F.)
AND *PIPER GUINEENSE* (THONN.) AGAINST
IMMATURE *CALLOSOBRUCHUS MACULATUS* (FAB.)
(COLEOPTERA : BRUCHIDAE) ON STORED COWPEA,
VIGNA UNGUICULATA WALP.

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ABSTRACT

Laboratory experiments were conducted to assess the insecticidal effects of seed powders of *Dennettia tripetala* (Pepper fruit) and *Piper guineense* (Black papper) on the egg, larva, and pupa of *Callosobruchus maculatus*. Results indicated that each of these peppers was effective in the control of *C. maculatus* at the egg stage. Virtually no emergence occurred when treatment of both peppers was applied within the first three days of oviposition. Beyond this period, significant emergence occurred. Results also indicated that these peppers were not effective during the larval and pupal stages. This is attributable to the fact that the larva and pupa develop within a single seed and are thus, shielded from the effects of these peppers. In contrast, the eggs are attached singly to the seeds. This study has, therefore, revealed that for the effective control of this pest, it is critical to apply these peppers before egg hatch; once the egg hatches and the larva bores into the seed, control is uncertain.

INTRODUCTION

Callosobruchus maculatus (Fab.) is the most important storage pest of cowpea throughout the tropics (Akingbohunge, 1976; Caswell, 1981; NRI, 1996). Infestation often occurs in the field when pods are close to maturity and usually originates from farm stores (Prevett, 1961).

The control of this pest had been effected through fumigation and with insecticides such as malathion and pirimiphos methyl (Caswell and Akibu, 1980; NRI, 1996). However, concerns have arisen about the persistence of insecticidal residues in stored products which can be harmful to mammals. These concerns are more acute in the developing countries where most of the farmers are illiterate and lack the technical knowledge to apply these chemicals according to the recommended concentrations. Institutions that regulate the use of these chemicals are neither efficient nor effective. As a result, these

countries have become recipients of fake, adulterated, expired and banned chemicals (Abate *et al.*, 2000). For instance between 1994 and 1997, FAO registered 10,099 metric tons of obsolete, unwanted, and/or banned insecticides in 34 African countries (FAO, 1998). In addition, these conventional chemical insecticides are expensive and not readily available to many peasant farmers in the developing world who store their grains mostly in clay pots and plastic containers (Caswell, 1976).

As a result of the problems associated with conventional chemical insecticides, researchers have been searching for alternate means of controlling these pests. One of these, is the use of plant extracts (Prates *et al.*, 1998). *Dennettia tripetala* (Bak. F.) and *Piper guineense* (Thonn.) have been reported as effective in the control of *C. maculatus* (Oji *et al.*, 1992; Mabata *et al.*, 1995), Gakuru and Foua-Bi, 1996; Okonkwo and Okoye, 1996).

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The adult *C. maculatus* does not feed but lays eggs attached to the pods or seeds and the damage is caused by the larvae feeding inside the seed (Singh *et al.*, 1990). The pest stage of *C. maculatus* is the larva and information on the effect of *D. tripetala* (pepper fruit) and *P. guineense* (Black pepper) on the immature stages of this pest is lacking. This information is essential in determining the critical stage of *C. maculatus* development for the application of these peppers to achieve the maximum effect.

This investigation was, therefore, designed to assess the effect of seed powders of *D. tripetala* and *P. guineense* on the egg, larva, and pupa of *C. maculatus*. *P. guineense* is widely distributed in West Africa, where it is traditionally used as a spice in flavouring food and in several formulations for the treatment of cough, gastro-intestinal disorders, cold, bronchitis, venereal diseases, and rheumatism (Dalziel, 1937).

MATERIAL AND METHODS

Laboratory experiments were conducted to assess the insecticidal effects of seed powders of *Dennettia tripetala* (pepper fruit) and *Piper guineense* (Black pepper) on the egg, larva, and pupa of *C. maculatus*. Seeds of ripe pepper fruits, purchased from a local market, were removed from the fruits, washed and sun-dried. The seeds were then milled, sieved, and weighed into batches of 1g, 2g and 3g and kept in airtight Kilner jar. Similarly, the seeds of black pepper were sun-dried, milled, and weighed into batches of 1g, 2g, and 3g and kept in airtight Kilner jar.

Effect of *D. tripetala* and *P. guineense* on *C. maculatus* eggs: Brown cowpea seeds, *Vigna unguiculata*, purchased from a local market, were disinfested, using cold shock treatment for four days at 0 to 4°C. Twenty-five grams of disinfested cowpea seeds were placed in each of 84 Kilner jars. Ten males and 15 females of newly-emerged *C. maculatus*

were introduced into each of the 84 Kilner jars containing the disinfested cowpea seeds. After 24 hours, all adult *C. maculatus* were removed from each of the 84 Kilner jars. Twelve of these jars were then set aside, divided into three replicates of four jars per replicate and treated with the weighed seed powder of pepper fruit. The treatments were 1g, 2g, 3g and 0g pepper fruit/25g cowpea (control). Subsequently, at 24-hour intervals, 12 of the 84 jars were set aside, replicated, and treated as described above until the 84 jars were exhausted in seven days. The jars were made airtight, kept under fluctuating laboratory conditions (20-30°C and 70-85% r.h.) and monitored for adult emergence. Similar trials were undertaken with black pepper. The number of adults that emerged from each treatment was counted and recorded.

Effect of *D. tripetala* and *P. guineense* on *C. maculatus* larvae: Twenty-five grams of disinfested cowpea were placed in each of 12 Kilner jars. Ten males and 15 females of newly-emerged *C. maculatus* were introduced into each of the 12 jars. After 24 hours, the adults were removed from all the jars. The jars were then retained for 11 days to accommodate complete egg hatch. The jars were then divided into three replicates of four jars per replicate and treated with seed powder of pepper fruit. The jars were made airtight, kept under fluctuating laboratory conditions and monitored for adult emergence. Similar trials were conducted with the black pepper. After emergence, the adults in each treatment were counted and recorded.

Effect of *D. tripetala* and *P. guineense* on *C. maculatus* pupae: A procedure, similar to that used in determining the effect of the peppers on the larva was followed, except that after the removal of the adults at the end of 24 hours the jars were left for 21 days before treatment with the peppers. This was to allow for development to the pupal stage. After

treatment, the jars were made airtight, kept under fluctuating laboratory conditions and monitored for adult emergence. The emerged adults in each treatment were counted and recorded.

Data Analyses: Since the data involved insect counts, the number of adults from each treatment was subjected to square root transformation (Sokal and Rohlf, 1981). All the data were then subjected to Analysis of Variance. Those data in which the analysis of Variance indicated significant differences among the treatment means, were subjected to either Least Significant Difference (LSD) (Steel and Torrie, 1980) or Student-Newman-Keuls (SNK) Procedure (Gomez and Gomez, 1984) depending on the number of treatment means.

RESULTS AND DISCUSSION

Variation in adult *C. maculatus* emergence from eggs on cowpea treated with *D. tripetala* and *P. guineense*: The means of adult emergence were 0.00±0.00, 0.00±0.00, 0.00±0.00, 19.81±66.03, 20.31±63.44, 22.92±99.25 and 43.63±111.47 from day one to day seven of treatment with *D. tripetala*, respectively and 0.00±0.00, 0.00±0.00, 0.49±2.92, 14.99±28.17, 25.01±48.27, 37.28±8.73, 68.79±48.33 from day one to day seven of treatment with *P. guineense*,

respectively (Table 1). Analysis of Variance indicated that there were significant differences among the mean emergence over the seven days in both *D. tripetala* and *P. guineense*-treated samples. Student-Newman-Keuls Procedure indicated that in both peppers, a significantly higher number of adults emerged when samples were treated seven days after oviposition compared with samples treated 1-6 days after oviposition (Table 1). No adult emergence occurred when samples were treated with *D. tripetala* on the first, second, and third days after oviposition, and on the first and second days after oviposition, when samples were treated with *P. guineense* (Table 1).

The means of adult emergence under different pepper concentrations were 85.56±1.06, 18.58±10.96, 18.40±13.54 and 1.28±1.08 for the control, 1g, 2g and 3g *D. tripetala*/25g cowpea, respectively while for *P. guineense* the corresponding means were 81.54±0.66, 13.25±7.13, 16.08±8.94 and 10.76±7.45 (Table 2). Analysis of Variance indicated that there were significant differences among the means in both pepper trials. Fisher's (Protected) Least Significant Difference indicated that in each pepper trial, all the three treatments significantly reduced adult emergence in contrast to the control (Table 2).

Table 1. Timing of application on the insecticidal effects of seed powders of *Dennattia tripetala* and *Piper guineense* on cowpea* during the egg stage of *Callosobruchus maculatus*

| Days** | Mean number of emerged adults on <i>D. tripetala</i> treated samples (±95% Confidence limit) | Mean number of emerged adults on <i>P. guineense</i> treated samples (±95% Confidence limit) |
|--------|--|--|
| 1. | 0.00a±0.00 | 0.00a±0.00 |
| 2. | 0.00a±0.00 | 0.00a±0.00 |
| 3. | 0.00a±0.00 | 0.49a±2.92 |
| 4. | 19.81b±66.03 | 14.99b±28.17 |
| 5. | 20.31b±63.44 | 25.01c±48.27 |
| 6. | 22.92b±99.25 | 37.28d±8.73 |
| 7. | 43.63c±111.47 | 68.79e±48.33 |

Means represent values from these replicates.

Means followed by the same alphabets are not significantly different from each other ($P < 0.05$; Student-Newman-Keuls Procedure).

* Twenty five grams of cowpea were used in each treatment.

** Period between oviposition and treatment with *D. tripetala* and *P. guineense*.

Table 2. Insecticidal effects of different concentrations of seed powders of *Dennettia tripetala* and *Piper guineense* on cowpea, during the egg stage of *Callosobruchus maculatus*

| Seed powder | Weight of seed powder (g) | Mean number of emerged adults ($\pm 95\%$ Confidence limit) |
|---------------------|---------------------------|--|
| <i>D. tripetala</i> | 0 | 85.56a \pm 1.06 |
| | 1 | 18.58b \pm 10.96 |
| | 2 | 18.40b \pm 13.54 |
| | 3 | 1.28c \pm 1.08 |
| <i>P. guineense</i> | 0 | 81.54a \pm 0.66 |
| | 1 | 13.25b \pm 7.23 |
| | 2 | 16.08b \pm 8.94 |
| | 3 | 10.76b \pm 7.45 |

Means represent values from these replicates.

Means followed by the same alphabets are not significantly different from each other ($P < 0.01$, Fisher's (Protected) Least Significant Difference).

Table 3. Insecticidal effects of different concentrations of seed powders of *Dennettia tripetala* and *Piper guineense* on cowpea, during the larval stage of *Callosobruchus maculatus*

| Seed powder | Weight of seed powder (g) | Mean number of emerged adults ($\pm 95\%$ Confidence limit) |
|---------------------|---------------------------|--|
| <i>D. tripetala</i> | 0 | 84.82a \pm 39.02 |
| | 1 | 91.59a \pm 66.29 |
| | 2 | 81.36a \pm 25.39 |
| | 3 | 92.93a \pm 10.83 |
| <i>P. guineense</i> | 0 | 78.68a \pm 61.19 |
| | 1 | 79.39a \pm 27.49 |
| | 2 | 78.68a \pm 44.51 |
| | 3 | 95.45a \pm 40.86 |

Means represent values from these replicates.

Means followed by the same alphabets are not significantly different from each other (Analysis of Variance).

Table 4. Insecticidal effects of different concentrations of seed powders of *Dennettia tripetala* and *Piper guineense* on cowpea, during the pupal stage of *Callosobruchus maculatus*

| Seed powder | Weight of seed powder (g) | Mean number of emerged adults ($\pm 95\%$ Confidence limit) |
|---------------------|---------------------------|--|
| <i>D. tripetala</i> | 0 | 75.34a \pm 72.95 |
| | 1 | 82.99a \pm 31.08 |
| | 2 | 80.82a \pm 19.73 |
| | 3 | 83.54a \pm 22.41 |
| <i>P. guineense</i> | 0 | 85.54a \pm 33.56 |
| | 1 | 77.26a \pm 6.26 |
| | 2 | 95.65a \pm 18.81 |
| | 3 | 81.72a \pm 61.63 |

Means represent values from these replicates.

Means followed by the same alphabets are not significantly different from each other (Analysis of Variance).

Variation in adult *C. maculatus* emergence from larvae on cowpea treated with *D. tripetala* and *P. guineense*. The means of adult emergence, when treatment was at the larval stage, were 84.22 \pm 39.02, 91.59 \pm 66.29, 81.36 \pm 25.39, and 92.93 \pm 10.83 for the control, 1g, 2g and 3g *D. tripetala*/25g cowpea, respectively, while

in *P. guineense*-treated samples, the corresponding means were 78.68 ± 61.19 , 79.39 ± 27.49 , 78.68 ± 44.51 and 95.45 ± 40.86 (Table 3). Analysis of Variance indicated that there were no significant differences among the treatments means in both pepper trials (Table 3).

Variation in adult *C. maculatus* emergence from pupae on cowpea treated with *D. tripetala* and *P. guineense*. The means of adult emergence from pupae on cowpea treated with *D. tripetala* were 75.34 ± 22.95 , 82.99 ± 31.08 , 80.82 ± 19.73 and 83.54 ± 22.41 for the control, 1g, 2g and 3g *D. tripetala*/25g cowpea, respectively, while in *P. guineense*-treated samples, the corresponding means were 85.00 ± 33.56 , 77.26 ± 6.26 , 95.65 ± 18.81 and 81.72 ± 61.63 (Table 4). Analysis of Variance indicated that there were no significant differences among the treatment means in both pepper trials (Table 4).

Results indicated that *D. tripetala* and *P. guineense* were effective in the control of *C. maculatus* at the egg stage. Virtually no emergence occurred when treatment of both peppers was applied within the first three days of oviposition. Beyond this period, emergence occurred. Results also indicated that these

peppers were not effective in the control of this pest, if applied during the larval and pupal stages of development. This can be attributed to the fact that the larva and pupa develop within a single seed (NRI, 1996) and are, therefore, shielded from the effect of these peppers. On the other hand, the eggs are attached singly to the seeds (NRI, 1996). This study has, therefore, revealed that for effective control of this pest, it is critical to apply these peppers before egg hatch. Once the egg hatches, and the larva bores into the seed, control is difficult.

Before the advent of conventional chemical pesticides in Africa, farmers used various forms of herbal and animal products in the control of pests and diseases but the active ingredients were not known and the products were not standardized (Abate *et al.*, 2000). Constituents of *P. guineense* include piperine (Smith, 1989) and dihydro piperine (Ojinnaka, 1987); both have shown antimicrobial activity and may also be responsible for the insecticidal property. The identification and characterization of *D. tripetala* and *P. guineense* constituents responsible for the insecticidal property are currently being investigated.

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