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# Competition in the Parasitization of *Callosobruchus maculatus* between *Dinarmus basalis* and *Choetospila elegans* on *Vigna unguiculata* (Walp.) Seeds

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## Abstract

Laboratory study was carried out to investigate the efficacy of hymenopteran parasitoids, (*Dinarmus basalis* and *Choetospila elegans)* parasitization of *Callosobruchus maculatus* on cowpea (*Vigna unguiculata*) seeds in the Department of Crop Science, University of Calabar, Nigeria. Treatments were the inoculation of *D. basalis* (sole), *C. elegans* (sole), a mixture of *D. basalis* and *C. elegans* into infested cowpea seeds and the control which received no parasitoids. The introduction of parasitoids was repeated at 3 d interval, and all treatments were replicated 3 times. Result indicated that early introduction of the parasitoids caused significantly ($P \leq 0.05$) higher mortality of *C.maculatus* than late introduction. Mix culture of both parasitoids was significantly more efficacious than sole culture. The emergence of each parasitoid and the mortality of *C. maculatus* in sole culture showed a highly significant ($P \leq 0.01$) positive relationship ($r = 0.487$) between *C. maculatus* and *D. basalis* but non-significant ($P \geq 0.05$) though positive association ($r = 0.289$) with *C. elegans*. A multiple regression analysis between *C. maculatus* mortality and the emergence of the two parasitoids in mix culture also indicated a highly positive significant ($P \leq 0.01$) relationship ($R = 0.751$, $R^2 = 0.564$). Pearson Correlation analysis also showed a significant ($P \leq 0.05$) positive association ($r = 0.464$, and 0.401) between *C. maculatus* mortality and emergence of *C. elegans* and *D. basalis* in mix culture, respectively. However, a non significant ($P \geq 0.05$) negative relationship ($r = -0.336$) was observed between the two parasitoids in their emergence in mix culture, but *D. basalis* contributed more to the mortality of *C. maculatus* than *C. elegans*. Data from this study showed that *D. basalis* and *C. elegans* have potentials to be deployed as biological control entities in the management of *C. maculatus* as a part of the integrated pest management strategies of storage pests in the tropics.

**Keywords:** parasitoids, *Callosobruchus maculatus*, *Choetospila elegans*, *Dinarmus basalis*, mortality

## 1. Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the most widely adapted, versatile and nutritious grain legumes, and has been consumed by humans since the earliest practice of agriculture in developing counties of Africa, Asia and Latin America, where it is especially valuable as a source of dietary proteins as well as vitamins and minerals (Singh et al., 2003; Langyintuo et al., 2003; Ukeh & Udo, 2008). Recent post harvest losses have been estimated at $5billion per year in the US (mainly due to insects and microbes which usually work in concert) with these being proportionally higher in developing countries (Haines, 1991).

The cowpea beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) is a tropical insect that develops in the seeds of *Vigna unguiculata* (L.) Walpers (Zannou et al., 2003). The insect is a field - to - store pest as its infestation of cowpea often begins in the field as the mature pods dry (Huignard et al., 1985; Haines, 1991). The insect multiplies very rapidly in storage where it causes very high losses (Oudraogo et al., 1996; Sanon et al., 1998). Okokon et al. (2004) reported that *C. maculatus* damage level to cowpea reached 50% after six months storage. According to IITA (1989) *C. maculatus* consumes 50-90% of cowpea in storage annually throughout tropical Africa. The bruchid infestation also affects seed quality, market value and can reduce cowpea seed viability to 2% after three months of storage (Ofuya & Credland, 1995; Ukeh et al., 2011). According to

Ouedraogo et al. (1996) the life cycle of *C. maculatus* takes about 4-5 weeks and about 6-7 generations are quite common in many countries. A female lays about 90-100 eggs in her lifetime. The adult insect lives for about 4-5 weeks. Species of natural enemies are associated with stored-product insects (Brower et al., 1996) and their potential as biological agents for these pests have been widely studied (Ouedraogo et al., 1996; Donnelly & and Philips, 2001; Amevoin et al., 2007; Iloba et al., 2007). In West Africa (Niger, Burkina Faso, Benin) the solitary ectoparasitoid *Dinarmus basalis* Rendani (Hymenoptera: Ptermalidae) and its sympatric species *Eupelmus vuilleti* Crawford and *E. orientalis* Crawford (Hymenoptera: Eupelmidae) parasitize the larvae and pupae of *C. maculatus* (F.) and *Bruchidius atrolineatus* (Pic) (Coleoptera: Bruchidae) which develop inside the seeds of *V. unguiculata* (Danielle Rojas-Rousse, 2010).

*Dinarmus basalis* and *C. elegans* have also been found together in cowpea infested with *C. maculatus* in the Nigerian markets and stores and their combined effects as parasitoids have not been investigated. This study was therefore designed to evaluate the efficacy of parasitism of the two parasitoids on *C. maculatus* in cowpea seeds.

## 2. **Materials and Methods**

### *2.1 Culturing of Insect Species*

Four kilogrammes of cowpea seeds collected from a local market in Calabar, Southern Nigeria were sterilized in an oven regulated at 60 °C for 24 hours according to Murdock and Shade (1987). The sterilized seeds were weighed into 120 g lots into 36 kilner jars covered with net meshed lids and the cowpea seeds were now re-infested with *C. maculatus* adults from the stock maintained in the Department of Crop Science, University of Calabar, Nigeria. The *C. maculatus* were allowed to lay eggs for five days before being removed with a sieve.

Cultures of the hymenopterous parasitoids (*D. basalis* and *C. elegans* were reared on infested and parasitized *V. unguiculata* seeds purchased from the market. *Callosobruchus maculatus* was separated into a tray with 2mm-mesh sieve for new infestation and an aspirator was used in selecting the newly emerged normal male and female *C. maculatus* and the parasitoids for experimental infestations. Twenty grams of disinfested *V. unguiculata* seeds were weighed into nine different specimen bottles covered with fine nylon net lids of about 1x1mm mesh size. The cowpea in the specimen bottles was then infested with ten pairs of the newly emerged *C. maculatus* and kept aside to lay eggs for three days after which they were removed. The total eggs per specimen bottle were recorded at the end of the oviposition period.

### *2.2 Infestation with Parasitoids*

After recording the total number of eggs laid, the bottles containing the infested cowpea were arranged into 9 sets (four groups in each set). The first group was assigned the following treatments: six pairs of *D. basalis* (sole culture) were introduced into one bottle, six pairs of *C. elegans* (sole culture) into the second, three pairs of *D. basalis* and three pairs of *C. elegans* into the third (mix culture) while the fourth bottle had no parasitoid and served as control. The exercise was repeated for the remaining eight sets but at three days intervals. All treatments were replicated three times and arranged as a 4 x 9 factor factorial in a completely randomized design (CRD) placed on a laboratory bench under room temperature (28± 2 º C and 60±5% RH and 12: 12 light: dark regime) until F1 progeny emerged. The experiment was monitored and observed for 38 days until no more *C. maculatus* emerged.

### 2.3 *Data Collection and Analysis*

The total number of eggs laid in each of the experimental bottle was recorded and subjected to analysis of variance (ANOVA). Daily emergence of adult *C. maculatus* was recorded and sieved off to prevent further mating and oviposition. The total emergence was then subtracted from the total number of eggs laid in each bottle to get the total mortality which was used in the analysis and was expressed as percentage mortality. Mortality was adjusted using Abbot's formula (1925). Analysis of variance was used to calculate the F value and the means separated using the Fishers least significant difference (Wahua, 1999). Regression analysis was done between the parasitoids that emerged and the mortality of *C. maculatus* and the best fit equation was predicted using the derived equation.

## **3. Results**

Data from this study showed that adult *C. maculatus* started to emerge at 20 days after oviposition while the parasitoids emerged from 12 days after inoculation in each experimental culture. There was no significant difference ($P \geq 0.05$) in the mean number of eggs laid in each of the culture bottles (Table 1).

Table 1. Mean* number of eggs laid in each culture bottle

| *Culture bottles* | *Control* | *D* | *C* | D+C |
|---|---|---|---|---|
| 1 | 183 | 166 | 208 | 189 |
| 2 | 217 | 167 | 206 | 174 |
| 3 | 207 | 176 | 197 | 219 |
| 4 | 163 | 176 | 173 | 168 |
| 5 | 206 | 157 | 189 | 106 |
| 6 | 209 | 203 | 197 | 116 |
| 7 | 159 | 175 | 188 | 207 |
| 8 | 197 | 204 | 183 | 147 |
| 9 | 201 | 193 | 117 | 158 |

LSD (0.05) = Not significant.

*Mean of three replicates.

*Mortality of C. maculatus due to the parasitoids*

The results (Table 2) showed that there were significant ($P \leq 0.05$) differences in the percentage motality due to parasitism by the different parasitoids. *Dinarmus basalis* and *C. elegans* in mix culture resulted in significantly ($P \leq 0.05$) more mortality of *C. maculatus* than each of the sole cultures. Except at 3 d after inoculation, *Dinarmus basalis* in sole culture also resulted in significantly ($P \leq 0.05$) more mortality than its *C. elegans* counterpart. However, the least mortality was observed on the control culture. Early introduction of parasitoids also caused a significantly ($P \leq 0.05$) higher mortality of *C. maculatus*. The least mortality was observed in the control (Table 2).

Table 2. Effects of time of introduction of parasitoids on the mortality of *Callosobruchus maculatus* on stored cowpea seeds

| Time of inoculation(days) | Parasitoids | | | | |
|---|---|---|---|---|---|
| | Control | C | D | D+C | Time mean |
| 3 | 12.06 | 85.76 | 81.71 | 84.64 | 66.04 |
| 6 | 6.60 | 79.97 | 75.67 | 84.22 | 61.62 |
| 9 | 10.97 | 71.00 | 70.40 | 86.94 | 59.83 |
| 12 | 6.65 | 68.17 | 70.96 | 83.99 | 57.44 |
| 15 | 7.21 | 64.82 | 67.90 | 81.29 | 55. 31 |
| 18 | 3.99 | 49.54 | 54.82 | 62.88 | 42.81 |
| 21 | 4.47 | 39.90 | 44.95 | 50. 78 | 35.03 |
| 24 | 9.01 | 25.74 | 36.39 | 39.60 | 27.69 |
| 27 | 11.58 | 14.14 | 21.07 | 27.33 | 18.53 |
| | 8.06 | 55.45 | 58.21 | 66.85 | |

LSD (0.05) for Time of inoculation = 2.18.

LSD (0.05) for parasitoid =1.46.

LSD (0.05) for interaction (time x parasitoid) = 4.37.

D = *Dinarmus basalis*

C = *Choetospila elegans*

D + C = *Dinarmus basalis* and *Choetospila elegans.*

A multiple regression analysis between *C. maculatus* mortality as the dependent variable and the emergence of the two parasitoids as the independent variables in mix-culture indicated a highly positive significant ($P \leq 0.01$) relationship (R=0.751) between these variables. About 56% ($R^2= 0.564$) of the variation was accounted for by the regression. The emergence of *D. basalis* accounted more for *C. maculatus* mortality than of *C. elegans* as depicted by the regression parameters as shown on the equation ($Y=22.509 +2.687X_1+1.027X_2$). Y predicted the mortality of *C. maculatus* with respect to the emergence of the two parasitoids in mix- culture. Thus, a unit increase in the emergence of *D. basalis* in mix culture with *C. elegans* caused about 2.7 times increase in *C. maculatus* mortality compared to 1.0 time increase by *C. elegans*. However, Pearson Correlation analysis showed a significant ($P \leq 0.05$) positive association (r = 0.464, and 0.401) between *C. maculatus* mortality and emergence of *C. elegans* and *D. basalis* in mix culture, respectively. More so, a non significant negative ($P \geq 0.05$) relationship (r=-0.336) was observed between the two parasitoids in their emergence. The relationship between each parasitoid in mixture against *C. maculatus* mortality is depicted graphically in Figs 1 and 2. The regression analysis between the emergence of each parasitoid and the mortality of *C. maculatus* in sole culture of the parasitoid showed highly significant ($P \leq 0.01$) positive relationship (r = 0.487) between the mortality of *C. maculatus* and emergence of *D. basalis* but non-significant ($P \geq 0.05$) though positive association (r = 0.289) with *C. elegans*. From the computed regression equations (i.e. Y=29.746 + 1.349X; Y=43.98 + 0.996X), each unit increase in the emergence of *D. basalis* and *C. elegans* resulted in 1.35 and 0.99 times increase in the mortality of *C. maculatus*, respectively.

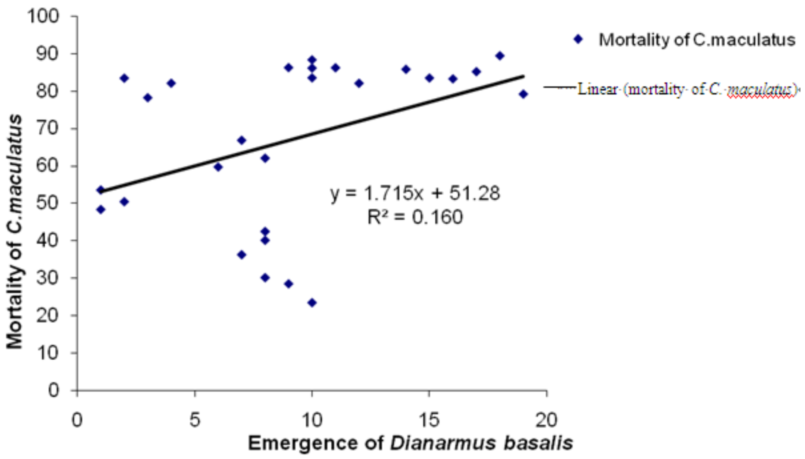

Figure 1. Mortality of *Callsobruchus maculatus* and the emergence of *Dianarmus basalis*

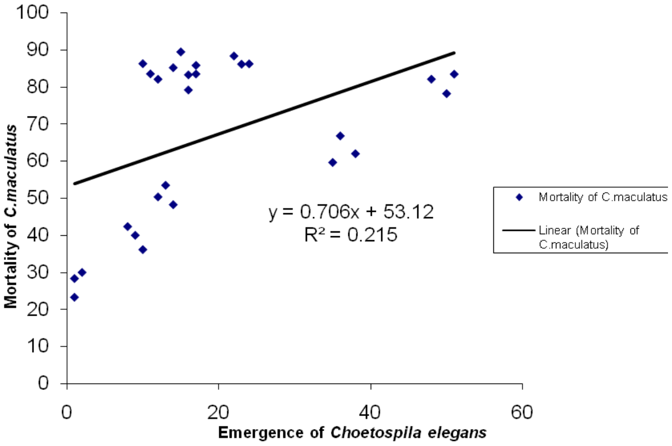

Figure 2. Mortality of *Callasobruchus maculatus* and the emergence of *Chetospila elegans*

## 4. Discussion

This research has confirmed reports by Ketoh et al. (2002), Iloba et al. (2007), Rojas-Rousse (2010) that *Dinarmus basalis* and *Choetospila elegans* reduce the population of bruchids in stores and therefore has the potential for utilization as a biological control agent against a serious internal-feeding pest of small grains, *C. maculatus* in a traditional storage bin. The time of introduction of the parasitoid wasps was very crucial in arresting the vulnerable stages of the bruchids which are the larval, the pre-pupa and the pupal stages. The introduction of parasitoids from three to six days (when the eggs are presumed hatched (Ouedraogo et al., 1996) to 20 days after the grains were infested apparently allowed for the proper life stage (early and late instar larvae) to be present for parasitization, and gave the parasitoid population a boost compared to the growth rate of the pest population. This study has also showed that the presence of more than one parasitoid in mixture gave more efficient control of the bruchids in the local bin than when one parasitoid species was present. Although there is no report in literature of the occurrence of *D. basalis* and *C. elegans* together elsewhere in the local granaries, this research has confirmed their presence in granaries in some of the Nigerian markets. Another solitary ectoparasitoid, *Pteromalus cerealellae* (Ashmead) (Hymenoptera: Pteromalidae), has also been recovered from the larvae of various stored-product insect pests. The known hosts of *P. cerealellae* include *Sitotroga cerealella* Olivier (Lepidoptera: Gelechidae), *C. maculatus*, the anobiid (*Lasioderma serricorne* (Fab.)), the bostrichid (*Prostephanus truncatus* (Horn)), and grain weevils (*Sitophilus* spp.) (Wen & Brower, 1994; Onagbola & Fadamiro, 2008). Recent investigations have opened perspectives for the use of these parasitoid wasps in both cowpeas stores.

In conclusion, a number of larval parasitoids such as *D. basalis*, *C. elegans* and others are frequently found in traditional granaries which are not treated with chemical insecticides. Their impact can be considerable and they should be taken into account in integrated pest management concepts for small scale storage.

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