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G MORPHOMETRIC ANALYSIS OF THE DEVELOPMENTAL STAGES AND INSECTICIDAL EFFICACY OF THREE BOTANICAL OILS AGAINST ADULT CALLOSOBRUCHUS ANALIS

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ABSTRACT

The developmental stages of *Callosobruchus analis* were observed under laboratory conditions at 28 \pm 2 °C and 72 \pm 5% relative humidity. The results showed that female C. *analis* began to lay eggs within 24 hours and have an oviposition period of 8.50 \pm 0.70 days and an average of 13.00 \pm 0.50 eggs were laid by individual *C. analis* throughout their lifetime. The mean developmental periods from egg to larva and larva to pupa were observed to be 8.50 \pm 0.79 and 4.50 \pm 0.70 days, respectively. The unmated bruchids were observed to have lived longer than the mated bruchids as the unmated bruchids lifespan was on average 10.50 \pm 0.81 days, as opposed to 2.50 \pm 0.75 days for mated females. Adult male C. *analis* have an average antenna length of 2.96 \pm 0.08 mm which is slightly longer than 2.42 \pm 0.12 mm on average for female bruchids. The three botanical oil extracts from *Capsicum frutescens*, *Anacardium occidentale* and *Xylopia aethiopica* used at 10.0% concentration were not effective, as none of them resulted in a mortality rate of 50% when recorded 3 days after treatment.

Key words: morphometric records, mortality, plant oils

INTRODUCTION

The bean weevils belonging to genus Callosobruchus are regarded as a primary pests of stored seeds of cowpea, pigeon pea and African yam bean. It also affects soya bean and groundnut (Dobie et al. 1984). They has caused a reduction in yield of over 80% in Nigeria (Oparaeke et al. 2000). The adult bruchids of bean weevils are less injurious to the stored seeds since they do not feed on them: rather they are a nuisance, thereby compromising the purity of the seeds. The larvae of Callosobruchus spp. are destructive because they bore holes into the seeds in an attempt to feed on the germ layer of the seed (Jackai & Dauoust 1986). The proliferation of Callosobruchus spp. is very rapid thereby causing much damage over a short period of time. Culturing these bruchids is quite easy in the laboratory and the biology of C. analis has been investigated extensively under laboratory conditions (Messina 1989).

In an attempt to control *C. analis*, several aspects of the biology of the bruchid have been studied and different plant oils employed. Nonetheless, the destructive activity of this bruchid is still pronounced, hence this research. Our study was aimed at investigating the duration of the developmental stages of *C. analis*, the morphometric measurements of some of the developmental stages, and the possible effectiveness of three plant (*Capsicum frutescens* (pepper), *Anacardium occidentale* (cashew nut seeds) and *Xylopia aethiopica* (Ethiopian pepper)) oil extracts in minute quantities for the control of *C. analis*.

MATERIALS AND METHOD

Bruchid culture

The *C. analis* used for this study was obtained from infested cowpea seeds bought at the local market. A large number of the bruchids were cultured in the laboratory (reared) in plastic containers of 1 liter in volume. The plastic containers were covered with muslin cloth and regularly fed with cowpea, under the ambient laboratory conditions of 28 ± 2 °C and $72 \pm 5\%$ relative humidity.

Yellow cowpea seeds, free of bruchids, were disinfested at -2 °C for 72 h. The disinfested seeds were kept safe until use.

Collection and preparation of plant material

C. frutescens, *A. occidentale* and *X. aethiopica* used in the experiment were obtained at a local market. The plants were air-dried, pulverized using a Muchang grinder, and then soaked in ethanol (extracting solvent) for a day after being stirred properly. The mixture was then filtrated using filter paper. The oils in the filtrates were then extracted using Soxhlet oil-extracting apparatus for 3 hours. The resulting oil extracts contained both extracting solvent and the oils, therefore they were exposed to air to remove traces of volatile ethanol.

Fecundity and oviposition

Two male and two female *C. analis* individuals were placed into a plastic container containing 1 g of cowpea seeds. The lids of the plastic containers were perforated with a pin to allow aeration. The activities of the insects whilst mating were studied. The procedure was triplicated for each number of days. The number of eggs laid was counted each day and the average number of eggs was estimated. **Measurements, hatching of eggs and the periods of metamorphosis of the eggs to adulthood**

Using a microscope fitted with an eyepiece micrometer the following measurements were made: the size (length and breadth) of some of the laid eggs, the length and weight of the insects, and the lengths of the antennae of both male and female bruchids.

Twenty newly laid eggs on cowpea seeds were kept in a plastic container with a perforated lid and the developmental periods of eggs to larvae and larvae to adulthood were closely monitored using a microscope.

Control of adult *C. analis* using oil extracted from plants

Two concentrations, 5% and 10%, of the extracted oils in ethanol were prepared and used as protectants on the cowpea seeds. Ten grams of the disinfested cowpea seeds were placed into a plastic container of 100 ml in volume in triplicate and treated with 1 ml of three oil extracts at concentrations of both 5% and 10%. Control treatment (seeds not treated with oil but with ethanol) were also triplicated. Twenty adult *C. analis* (10 males and 10 females) of between 0 and 24 hours in age were introduced into the containers with both treated and untreated beans. The adult mortality rate was counted after 4 days and the cumulative mortality rate was determined.

Statistical Analysis

All data were analyzed using Analysis of Variance (ANOVA) at a confidence level of 95% which was carried out by the Statistical Package for the Social Sciences (SPSS). The means were separated using Duncan's new Multiple Range Test. This was conducted to determine if there were significant differences among the rates of mortality at different concentrations and time intervals.

RESULTS

Mating and female fecundity

Mating between adult male and female *C. analis* occurred both during the day and at night and started shortly after they were introduced into the new environment. The genital organs of the bruchids were held together during the process of mating. The male bruchid mounts the female but does not restrict their movement as both were found moving from one place to another.



Fig. 1. Fecundity of *C. analis*. The period of oviposition was extended to the 10^{th} and 11^{th} days for only a few of the *C. analis* observed. An average of 13 eggs was laid by female *C. analis* throughout their lifetime.

Developmental stage	Days	Range of days			
Egg	7.50 ± 0.70	7–8			
Larva	8.50 ± 0.79	8-12			
Pupa	4.50 ± 0.79	5–6			
Total	20.50 ± 0.97	20–26			
Mated male (adult) life span	2.00 ± 0.71	1–3			
Mated female (adult) life span	3.00 ± 0.79	1–5			
Unmated male (adult) life span	9.00 ± 0.70	8-10			
Unmated female(adult) life span	12.00 ± 0.92	8-15			
Each value is the mean \pm standard error of twenty replicates.					

Table 1. Developmental period (in days) of *C. analis* in/on cowpea (*Vigna unguiculata*)

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Table 2. Morphometric measurements of C. analis

Parts measured	Length (mm)		
Egg length (pole to pole)	0.58 ± 0.00		
Egg width (side to side)	0.34 ± 0.00		
Adult male antennae	4.40 ± 0.00		
Adult female antennae	5.80 ± 0.00		
Adult male	2.96 ± 0.08		
Adult female	2.42 ± 0.12		

The results of the fecundity show that female *C. analis* started laying eggs within 24 h after mating and the average number of eggs laid per day decreased as the bruchid aged (Fig. 1). The maximum and minimum number of eggs laid per day was observed in Days 1 and 8, respectively. Laid eggs were found glued to the seeds.

Developmental periods of *C. analis* on/in cowpea seeds

The average developmental period of eggs was observed to be 7.5 days, while the developmental period of larvae lasted for an average of 8.5 days. The total developmental period of *C. analis* was observed to be 20.5 days on average. Unmated *C. analis* lived longer than the mated ones (Table 1).

Morphometric measurements of the developmental stages of *C. analis*

Table 2 shows the morphometric measurements of insects during some of the developmental stages. The length of the eggs was on average 0.58 \pm 0.00 mm, while their width was 0.34 \pm 0.00 mm on average. Furthermore, the antennae lengths of the female and male *C. analis* were on average 2.42 \pm 0.12 and 2.96 \pm 0.08 mm, respectively.

Table 3: Effect of extracted plant oils on the mortality rate of twenty C. analis individuals

Source of oil	Concentra-	Time after treatment				
	tion (%)	24 hours	48 hours	72 hours	96 hours	
Xylopia aethiopica	5.0	$1.67 {}^{\rm a} \pm 1.25$	$5.00^{b} \pm 2.39$	$5.00^{a} \pm 2.39$	$8.33^{\mathrm{a}}\pm3.75$	
	10.0	$0.00^{\mathrm{a}} \pm 0.00$	$6.67^{b} \pm 2.89$	$11.67^{b} \pm 3.75$	$15.00^{\text{b}} \pm 5.15$	
Anacardium occidentale	5.0	$11.67^{ab}\pm3.75$	$15.00^{a} \pm 5.54$	$20.00^{\mathrm{a}}\pm7.36$	$26.67^a\pm9.13$	
	10.0	$15.00^{\text{b}} \pm 4.27$	$25.00^b\pm6.57$	$30.00^{\text{b}}\pm8.90$	$31.67^b\pm9.21$	
Capsicum frutescens	5.0	$6.67^{a} \pm 3.54$	$18.33^a \pm 5.54$	$26.67^a\pm7.90$	$28.33^a \pm 7.71$	
	10.0	$13.33^b\pm4.54$	$23.00^{ab}\pm3.21$	$33.33^{ab}\pm6.25$	$35.00^{ab}\pm5.00$	
Control		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	

Each value is the mean \pm standard error of three replicates. Values indicated by the same letter for each plant do not differ significantly.

Note: The control experiment contained seeds that were only treated with ethanol.

Effects of extracted plant oils on the mortality rate of *C. analis*

The effects of various extracted plant oils on the mortality rate of *C. analis* at concentrations of 5% and 10% are shown in Table 3. None of the extracted oils regardless of concentrations killed more than 50% of the adult *C. analis* within 4 days following treatment. Although there was a progressive cumulative increase in the rate of mortality as the concentration and number of days following treatment increased, the rate of increase was very slow. Four days after the treatment with *X. aethiopica* oil only 8.33 and 15.00% were dead at oil concentrations of 5% and 10%, respectively. *A. occidentale* oil killed 26.7 and 31.7% of insects and *C. frutescens* killed 28.3 and 35.0% of insects at oil concentrations of 5 and 10%, respectively.

DISCUSSION

Copulation of *C. analis* started shortly after the emergence of adult *C. analis*. An average of 13 eggs was laid by a female *C. analis* individual over its entire lifetime and the eggs laid were found glued firmly to the surface of the seed. A similar observation was reported by Janzen (1977). The morphology of the eggs observed showed that the eggs of *C. analis* are grey and dome-shaped with an oval and flattened base. A similar observation was observed by Southgate (1978).

The incubation period of the eggs of *C. analis* was observed to be 7.50 ± 0.79 days on average. The larvae are whitish in color and apodous. The larvae feed entirely within a single seed, excavating a chamber within the cotyledon as they grow. A similar finding was reported by Southgate et al. (1957). The adult *C. analis* emerged from the seeds after an average of 20.50 ± 0.95 days. This finding was similar to an experiment by Southgate et al. (1957) who reported that the total developmental period of *C. analis* is 28 days. This reduction in the developmental period of *C. analis* might be a result of environmental factors.

Moreover, these studies showed that unmated females of *C. analis* live longer than mated ones. This finding coincides with the report by Ashamo and Akinneye (2004) which states that the average lifetime of unmated female *Euzopherodes vapidella* is longer than that of male *E. vapidella*. This variation in lifespan may be due to the fact that much energy is expended during mating.

The plant oils of *C. frutescens*, *A. occidentale* and *X. aethiopica* used for the control of *C. analis* were only slightly effective against *C. analis* as the mortality rate recorded 96 h after treatment ranged from 8.33 to 35.00%. The effectiveness of the *A. oc-cidentale* and *C. frutescens* oils was higher than that of *X. aethiopica* oil. Concentrations in excess of 10% might prove more effective in the control of adult *C. analis* and should be studied in the future.

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