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Efficacy of bioart biocides on eggs and adults of *Callosobruchus maculatus* Fab, main pest of cowpea stocks

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Abstract
Cowpea (*Vigna unguiculata*) is an essential legume for agriculture and nutrition in Senegal. However, its seeds are often affected by *C. maculatus* infestations. The objective of this research is to evaluate the biocidal effectiveness of BioArt, a natural biopesticide, against *Callosobruchus maculatus*, the main pest of cowpea stocks in Senegal. The experiments carried out at the Entomology and Acarology laboratory at Cheikh Anta Diop University in Dakar focused on the white cowpea variety, where ovicidal, larvicidal and adulticide tests were carried out. The results show total adult mortality in just six days for high concentrations (C1 and C2) and 100% larval mortality for intermediate and low concentrations (C2 and C3). Furthermore, the product significantly reduces the fecundity and fertility of treated females, thus disrupting their reproductive cycle. These results confirm the potential of BioArt as an ecological alternative to chemical pesticides, limiting environmental impacts while effectively protecting cowpea crops.

Keywords: *Callosobruchus maculatus*, bioart, *Vigna unguiculata*, biocidal effects

Introduction

Belonging to the Fabaceae family, cowpea *Vigna unguiculata* (L.) Walp is a seed legume native to Africa, now cultivated in almost all tropical and subtropical regions. It constitutes the most important grain legume crop in sub-Saharan Africa, particularly in arid to semi-arid savannah areas. The main world producers are Nigeria and Niger, which between them account for almost half of global production.

Cowpea plays a crucial role in food security and the rural economy in West Africa. With a protein content greater than 20%, its ripe seeds represent a valuable source of amino acids. They also contain a high proportion of starch (50 to 67%), abundant dietary fiber, and B vitamins, such as pantothenic acid or folic acid. In addition, the seeds are rich in essential trace elements, including iron, calcium and zinc, while having a low fat content, making them a food of great nutritional value. However, cowpea production and storage are seriously compromised by *Callosobruchus maculatus*, a pest beetle capable of destroying up to 100% of stocks in a few months (Thiaw, 2008) [16]. This insect attacks the energy reserves contained in the cotyledons of seeds, thus causing their rapid deterioration (Huignard *et al.*, 2011) [8]. Traditional control methods, although quite widespread, often prove ineffective in the face of massive infestations. Furthermore, the use of chemical pesticides, although frequently effective, presents major disadvantages: risks for human health and the environment, as well as the appearance of resistance among pests. This damage pushes some authors to use plant extracts to fight against these pests (Fall *et al.*, 2024 [2]; Faye *et al.*, 2014 [4]; Mbaye *et al.*, 2014) [13]. In this context, the development of biopesticides such as BioArt appears to be a promising alternative. The replacement of synthetic pesticides and antimicrobials with plant extracts is a current alternative adopted by traditional and family farmers and many pioneers of organic agriculture (Mahrach *et al.*, 2021) [10]. This study focuses on the Dakar strain, evaluating the effectiveness of BioArt through various tests targeting eggs (ovicidals), larvae (larvicidals) and adults (adulticides). The results obtained aim to guide sustainable management strategies for cowpea pests.

Material and method

We used glass jars with perforated lids, ensuring adequate aeration for the cowpea specimens and seeds, key elements of our research. Plastic boxes with perforated lids are also used for different phases of investigations. For precise manipulations, we use beakers, micropipettes and forceps, guaranteeing a methodical and precise approach. Additionally, a monocular magnifier is essential for detailed observations, deepening our understanding of cowpea specimens and seeds.

The plant material used for mass breeding and experiments included cowpea seeds (*Vigna unguiculata* L. WALP purchased at the Castor market (Dakar). These seeds were transported to the laboratory, placed in glass jars and kept in the freezer for 96 hours to eliminate any hidden infestation. Then, they were brought to room temperature and placed in tightly closed jars 16 cm high and 8 cm wide. diameter. Cowpea bruchids (*C. maculatus*) were selected as animal material, sampled from a batch of already infested cowpea seeds, coming from the entomology and acarology laboratory.

The adults are placed in glass jars 16 cm high and 8 cm in diameter, with perforated lids for optimal ventilation. A quantity (250g) of healthy cowpea seeds is added to each jar. The jars are left at room temperature. After 24 hours of contact with bruchids, allowing mating and oviposition, seeds are removed and placed in rubber boxes with perforated lids, and monitored until adults emerge. An inspection of emerged adults is carried out every 24 hours to avoid mixed generation batches. Adults less than 48 hours old are recovered and used for experiments or for strain maintenance.

Experimental protocol for tests with BioArt
Three solutions of distinct concentrations were prepared according to the following procedure:
C1: 2 ml of the initial BioArt solution,
C2: 2 ml of C1 diluted with 2 ml of well water,
C3: 2 ml of C1 diluted with 4 ml of well water.

Ovicidal tests
As part of the experimental protocol, adults of C. maculatus aged 48 hours, from mass rearing, are paired in pairs (6 pairs) and placed in contact with healthy cowpea seeds in rubber boxes 6cm in diameter and 4cm in height. After 24 hours, the adults are removed and the seeds are observed under a monocular microscope to detect the presence of eggs. In the event of multiple eggs on the same seed, only one is kept, the others being removed with flexible forceps to avoid larval competition. Each box contains 12 seeds, each carrying an egg. These seeds are sprayed with 0.5 ml of the three concentrations of the BioArt solution, ensuring uniform distribution. Three repetitions and six controls (3 blanks and 3 solvents) are carried out for each concentration. No treatment is applied to the blank controls, while 0.5 ml of well water is sprinkled on the solvent controls. The boxes are placed on the laboratory benches at room temperature. After emergence of adults from the rescued eggs, a count of unhatched eggs and dead larvae is carried out by crushing the seeds, thus allowing us to calculate the embryonic and larval mortality rate according to the following formulas:

$$\%\ \text{embryonic mortality} = \frac{\text{Number of unhatched eggs}}{\text{Total number of eggs}} \times 100$$

$$\%\ \text{larval mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae}} \times 100$$

The observed mortalities are then corrected using the Abbott formula (Abbott, 1925). This formula makes it possible to obtain the corrected mortality values as a percentage, considering the mortalities of the treated samples as well as those of the blank control. The Abbott correction adjusts the results for natural mortality that may occur in the blank control, which helps to more accurately quantify the effectiveness of the treatment compared to baseline conditions. Abbott's formula is expressed as follows:

$$\text{Corrected mortality}\ (\%) = \frac{(\text{Mortality of the trafficking sample} - \text{Mortality of the white control})}{100 - \text{Mortality of the white control}} \times 100$$

Seed monitoring is maintained until adults emerge from the rescued eggs. The latter, coming from eggs treated with different concentrations of BioArt, are carefully matched in pairs. These couples are then placed in contact with healthy seeds at the rate of one couple per box, thus creating natural conditions to evaluate their fecundity (capacity to produce eggs) and the fertility of the eggs produced (capacity to give birth to larvae). Careful daily monitoring is carried out every 24 hours, noting precisely the number of eggs laid, both on the seeds and on the wall of the box. This method makes it possible to quantify female egg laying and observe behavioral variations in egg laying depending on BioArt concentrations. At each observation, the infested seeds are meticulously replaced by healthy seeds, guaranteeing a continuous and unaltered evaluation of the reproductive cycle, until the death of the female. The eggs are systematically observed from their laying until the emergence of adults. This post-emergence monitoring makes it possible to analyze the sex ratio of emerged adults and to determine the lifespan of these rescued adults.
The number of eggs laid per female (N): this number represents the total weighted sum of eggs laid by a female throughout her life.

$$N = \frac{\text{Number of eggs laid per female rescued}}{\text{Total number of females}} \times 100$$

The emergence rate (ET): It is determined by establishing the ratio between the total number of adults emerged and the total number of eggs laid.

$$TE = \frac{\text{Number of adults emerged}}{\text{Total number of eggs laid}} \times 100$$

The sex ratio (R) which gives the percentage of females compared to all the offspring. Sexing is done by observing the last abdominal tergite which is curved in the male and elongated in the female. If the sex ratio is greater than 50% then the sex ratio is in favor of females. Otherwise it is favored by males. It is determined by the following formula:

$$R = \frac{\text{Number of females emerged}}{\text{Total number of individuals emerged}} \times 100$$

Adulticide tests
As part of the experimental procedure, adult individuals of *C. maculatus* from mass rearing were paired in pairs. A set of six couples of adults aged less than 48 hours were put in contact with healthy cowpea seeds. The study was conducted in rubber boxes, each box containing 12 cowpea seeds and 6 pairs of *C. maculatus*. These adults were exposed to three distinct concentrations of the BioArt solution. Three repetitions and six controls (3 blank controls and 3 solvent controls) were set up for each concentration. The 0.5 mL solution of each formulation was applied with a micropipette, followed by gentle shaking to ensure uniform distribution. No treatment was administered to adults in the blank controls, while those in the solvent control were exposed to 0.5 ml of tap water. Mortality monitoring was carried out with careful counts every 24 hours, over a minimum period of 15 days. Each observation made it possible to identify the deceased adults among the matched couples. The boxes were kept on the benches, with the entire experiment taking place at room temperature. The proportion of adults who died for each concentration of the solution tested is calculated as follows:

$$\text{Adult mortality rate} = \frac{\text{Number of dead individuals}}{\text{Total number of individuals}} \times 100$$

Statistical analyzes
Average calculations and creation of graphs were carried out with Excel 2013. Statistical analyzes were carried out with R 2024.04.2 software. Normality of data was assessed with the Shapiro-Wilk test. As the majority of variables were not normally distributed, the Kruskal-Wallis test was used to compare group means. The results are considered significant for $p < 0.05$ (Sokal, 1995). If there was a significant difference, Dunn's test was used to determine which groups actually differed from each other.

Fig 1: Adult of *Callosobruchus maculatus*

Results

Ovicidal and larvicidal effect

Embryonic mortality varies significantly depending on the experimental conditions. In C1, it reaches 92.84%. However, in C2, embryonic mortality decreases slightly, going to 85.73%, while remaining high. On the other hand, in C3, it increases significantly to reach 96.50%, making this low concentration the most effective. These results suggest that embryos are particularly sensitive to these experimental concentrations, with maximum sensitivity at C3.

Regarding larval mortality, a very different trend is observed. In C1, no larval mortality is recorded, which means that the larvae resulting from viable embryos survive completely. However, in C2 and C3, larval mortality is total, reaching 100%. These results show that C2 and C3 concentrations are extremely unfavorable for larval development.

Table 1: the effect of the BioArt product on the eggs and larvae of *C. maculatus*

Concentration	Mortality Embryonic %	Mortality Larval %
C1	92,84	0,00
C2	85,73	100,00
C3	96,50	100,00

Remanence of BioArt on eggs from *C. maculatus* survivors

In C1, the total percentage of descendants is 55.03%. This figure indicates that egg laying is possible in this condition, although it is not optimal. The sex ratio, which is 53.84%, shows a slight predominance of males, which could reflect a moderate imbalance in the distribution of sexes among the offspring. On the other hand, the C2 and C3 concentrations show a total absence of egg laying. No offspring are produced. The absence of sex ratio in these conditions naturally reflects this absence of offspring.

Table 2: the effect of the BioArt product on the fertility of eggs laid by rescued females

Concentration	Total number of descendants (%)	Sex-ratio%
C1	55,03	53,84
C2	00	00
C3	00	00

Adulticidal effect

The figure presents adult mortality (%) over a period of 15 days (D1 to D15) for three experimental concentrations: C1, C2 and C3. Each concentration shows distinct dynamics of mortality progression.

In C1, adult mortality increases progressively between D1 and D5. From D5, we observe a sudden increase in mortality, reaching 100% from D6.

The C2 concentration shows a more moderate initial progression of mortality between D1 and D5. However, between D5 and D7, a notable acceleration is observed, with a jump to around 90%. From D8, mortality reaches a plateau of 100%, signaling the complete elimination of adults.

In C3, mortality increases more slowly. Until D6, the evolution is progressive and less marked than in the other two conditions. Between D7 and D11, a stabilization phase appears, with mortality oscillating around 70 and 80%. However, from D12, mortality begins to increase again and ends up approaching 100% around D14 and D15.

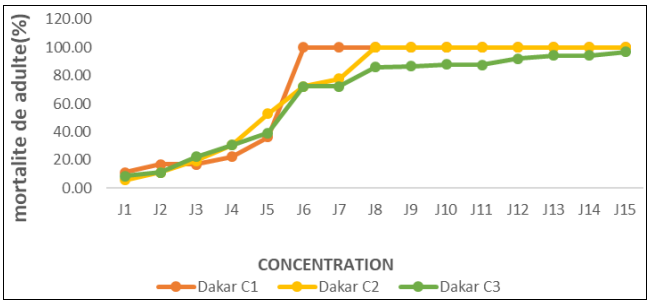

Fig 2: Adult mortality rate depending on concentration and duration of exposure

In C1, no individuals were produced, indicating a total absence of spawning. This situation reveals that the C1 concentration completely prevents egg laying in treated females. In C2, the total number of rescued individuals is 60.41%. This indicates that a significant portion of individuals survived despite the experimental conditions, although a notable proportion was affected. The sex ratio, which reaches 91.02%, reflects a marked imbalance in favor of one sex. In C3, the total number of rescued individuals is slightly higher, reaching 63.06%. The sex ratio, for its part, stands at 90.41%, also indicating a significant imbalance in favor of females, similar to that observed in C2. This trend suggests that C3 concentration influences sex ratio in a comparable manner to C2, although overall survival is slightly better.

Table 3: the effect of the BioArt product on the fertility of eggs laid by treated females

Concentration	Total number of individuals rescued (%)	Sex-ratio (%)
C1	Pas de ponte	Pas de ponte
C2	60,41	91,02
C3	63,06	90,41

Discussion

Data analysis reveals that the C1 concentration, although unfavorable for the embryos due to very high mortality (92.84%), remains favorable with the larval stage. On the other hand, C2 and C3 concentrations, in addition to presenting high embryonic mortality (85.73% and 96.50%), result in total incompatibility for larval survival (100%). The abrupt transition in larval mortality between C1 and C2 suggests a toxic effect or an environmental factor having a drastic impact on the viability of the larvae. Comparatively, the studies by Ketoh *et al.* (1998) [11] showed an elimination

of *C. maculatus* eggs of 70% with *C. schoenanthus* essential oil. Faye (2015) [3] reported embryonic mortality rates of 95.73% with neem leaves, 86.49% with *C. religiosa* leaves, and 90.95% with *S. occidentalis* leaves, while Ouali-N'goran *et al.* (2014) [14] demonstrated an emergence of 80.86% of adults on the IT97K499-38 variety.

Our results on the larvicidal effect differ from the larval mortality rates observed by Thiaw *et al.*, (2015) [18], which were 13.96 ± 4.85% with *S. occidentalis* extracts, and 4.17% and 5.63 ± 2.52% with hexane and acetate fractions on *C. serratus*. On the one hand, embryos could show a progressive sensitivity to concentration, while larvae show a more dichotomous response. On the other hand, specific environmental conditions, such as pH, the presence of toxic substances or temperature, could be responsible for the differences observed between the embryonic and larval stages. Finally, the biological mechanisms underlying the embryonic and larval stages could influence their respective tolerance to experimental concentrations.

The effects of BioArt on the fecundity of *C. maculatus* females show a significant reduction in egg laying. At concentrations C3 and C2, no mating was observed, resulting in a complete absence of fecundity. The C1 concentration reduces the clutch to 18.19±8.71 eggs, indicating a notable disruption of the reproductive cycle. The results agree with those of Gueye (2008) [6] concerning *Lantana camara* extracts, and with those of Thiaw *et al.* (2015) [18] concerning *Senna occidentalis* extracts on *Caryedon serratus* females.

Overall, the total number of offspring is greater than 50%, raising concerns about the resilience of *C. maculatus* populations. This survival rate is 55.03%. The results of Faye (2015) [3] on the same insect with extracts of *A. indica* contrast with our observations, while those of Dan Mairo (2011) [1] on *Boscia senegalensis* show higher fertility rates.

The sex ratio is in favor of males, which could intensify competition between males. BioArt also delays the duration of spawning-emergence.

The comparison of the three concentrations highlights different dynamics. C1 is the most toxic concentration, causing complete mortality of adults in a very short time (D6). C2 presents rapid but slightly more progressive mortality (D8). On the other hand, C3 shows delayed mortality with temporary resistance of adults before gradual elimination (96.9%) at the end of the period (D15). These results are consistent with those of Faye (2015) [3], who reported adult mortality of 87.25% with high concentrations C1 and C2 and 74.87% with C3 using aqueous extracts of *C. religiosa* powder on *C. maculatus* adults at day 10. Likewise, they confirm the conclusions of Seck *et al.* (1993) [15], who observed adult mortality of 80 to 100% with the application of leaves and fruits of *Boscia senegalensis* on *C. maculatus* and those of Thiaw (2004) [16] who revealed 100% adult mortality of *C. serratus* with extracts of *Calotropis procera*. The variable results of BioArt on adult *C. maculatus* could be explained by physiological differences between strains, the chemical composition of BioArt, and the natural resistance of the insect. Other factors could include interaction with the gut microbiome, synergistic or antagonistic effects with other substances, local adaptations and the developmental stage of the insect. The stabilization phase observed in C3 could reflect a temporary adaptation of adults, while the rapid increase in mortality in C1 and C2 reflects direct exposure to critical toxic thresholds.

BioArt showed a significant reduction in the fertility of treated females. In C1, the total absence of egg laying. On the other hand, the C2 and C3 concentrations allow the survival of a portion of the individuals, with survivor rates of 60.41% and 63.06% respectively. However, a marked imbalance in the sex ratio is observed, with a predominance of females. These observations allow us to formulate several hypotheses. The C1 concentration could act as a reproduction inhibitor, completely preventing egg-laying. C2 and C3 concentrations could have a moderate toxic effect, causing significant losses but still allowing partial survival. The imbalance observed in the sex ratio could be attributed to a differential sensitivity of the sexes to experimental conditions or to selection mechanisms favoring a particular sex. The work of Gueye (2008) [6] showed a reduction in the fecundity of females of *C. Serratus* with extracts of Lantana camara, while Kellouche and Saltani (2004) [10] reported that leaf powders of four plants rich in essential oils (fig, olive, lemon and eucalyptus) reduce the fecundity of females of *C. maculatus*, and that essential oils extracted from cloves completely inhibit egg laying. This observed reduction in fertility could be explained by the short lifespan of females, resulting from the biocidal effect of the product. Following application of the BioArt product to adults, it was observed that the insects were dead or paralyzed.

Conclusion

BioArt stands out as an ecological and sustainable solution to protect cowpea stocks from *C. maculatus*. By combining ovicidal, larvicidal and adulticidal action, it reduces agricultural losses while minimizing environmental risks. The high C1 concentration is more effective on adults. It is also necessary to note a remarkable ovicidal action leading to high egg mortality. The low C3 concentration is more effective on eggs. At the larval stage, C3 and C2 concentrations are more effective. BioArt also reduced egg laying in treated females and rescued adults. However, despite these encouraging results, the survival of eggs from treated females and rescued adults remains relatively high. Furthermore, the observation of an unbalanced sex ratio raises concerns about its impact on the insect's reproductive dynamics, requiring continued monitoring. Future research should explore the effectiveness of BioArt on other pests, as well as its large-scale application, in order to fully integrate this technology into agricultural practices in Senegal.

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