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Biocidal impact of *Carica papaya* leaves on adults and eggs of the legume pest *Caryedon serratus* (Olivier, 1790), pest of peanut stocks in Senegal

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Abstract: The three formulations (aqueous extract, fumigation and contact) based on *Carica papaya* leaves were tested on adults and eggs of *Caryedon serratus* in the Laboratory under ambient conditions. Contact with fresh leaves induced considerable adulticidal effects, spread over time depending on the doses applied ranging from 88.33% (10g) on the 7th day to 100% for high doses (15g and 20g) from the 4th day). It causes more or less significant and disproportionate egg mortalities to the doses applied, between 41.85% and 81.18%. Fumigation has a maximum effectiveness of 82.10% on adults with the application of all doses in different exposure periods depending on the doses applied (8 days (10g and 20g) and 9 days for 15g). It also gave embryonic mortalities of 67.61%. The aqueous extract of the powder of dry leaves of this plant (*Carica papaya*) induced an effectiveness proportional to the concentration applied on adults of *C. serratus*. This mortality varies depending on the duration of exposure (100% mortality with C1 from the 1st day, 97.82% mortality with C2 and C3 respectively on the 8th and 7th day). While the highest mortality of 51.08% was obtained with the C1 concentration on the eggs.

Keywords: *Caryedon serratus*, *Carica papaya*, aqueous extract, contact, fumigation, biocidal effects

1. Introduction

Caryedon serratus is one of the most harmful species, it attacks peanuts from the first week of drying of the pods in the fields. The first infestations are small at first, around 1 to 2%. The infestation continues and ultimately causes nutritional and economic impacts that vary depending on the initial level of the infestation, the destination of the production, the duration and the storage techniques. These losses become very serious from the second generation. Third, they are catastrophic, and generations follow one another for 2 to 3 months [1]. The damage observed can go up to 83% after 4 and a half months of storage in marketing centers [2]. In some villages, damage can even reach 100%. The attack of *Caryedon serratus* favors the spread of Aspergillus flavus, a fungus which produces aflatoxin, a carcinogenic substance. The stock therefore becomes soiled and unfit for consumption. In addition, losses in dry weight are recorded as well as other negative effects such as: the reduction in the market value of infested seeds, their nutritional value and that of their germinative ability. In fact, synthetic insecticides cause harmful effects on the health of populations as well as on the environment. Their excessive use, lack of precaution in their handling and failure to respect waiting periods are responsible for the resistance of certain harmful strains, the elimination of natural enemies, health problems (consumers and farmers) and environmental pollution. Faced with these disadvantages, scientists are looking into research into new methods such as the use of plants which have pesticidal properties against insect pests. 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) [3]. Several research have been carried out on the fight against cowpea pests with the use of plant extracts [4, 5, 6, 7]. It is in this context that we tested several formulations (contact, fumigation and aqueous extract) based on *Carica papaya* leaves to limit the damage of *Caryedon serratus* on peanut seeds.

2. Material and Methods

Biological material

The strain of *C. serratus* used in the experiment comes from peanut pods coming from the Diourbel region (Bambey/Thiakhar). The plant organs used are peanut seeds and papaya leaves. The peanut seeds which served as a food substrate for the insects, were purchased at the beaver market (Dakar), brought back to the laboratory and put in glass jars after a 96-hour stay in a refrigerator to eliminate a possible infestation. Papaya leaves are harvested early in the morning at the Botanical Garden of the Department of Plant Biology (FST /UCAD/Senegal).

Mass breeding

Breeding of the *C. serratus* insect was necessary to obtain a large number of individuals available for testing. Specimens of *C. serratus* are introduced into perforated plastic boxes 6.5 cm in diameter and 4.5 cm in height containing peanut seeds. After 24 hours of contact with the seeds, the bruchids were recovered. Infested seeds were monitored and the adults that emerged were used either for adulticide testing or to maintain mass breeding. The sexes are identified as follows: *Caryedon serratus*: Male, last abdominal tergite entirely covered by the elytra; Female, last abdominal tergite not covered by elytra.

Procedure for biocidal tests of *Carica papaya* on adults and eggs of *C. serratus*:

Contact with crushed fresh leaves

Carica papaya leaves used for contact testing are harvested early in the morning before sunrise to keep the substances active. They are crushed using a blender and put in small plastic boxes 6.5 cm in diameter and 4.5 cm in height. We used different weights (10g, 15g and 20g). The ground material thus obtained is placed in contact with the adults of *C. serratus* numbering 12 individuals per box. All the tests are repeated three times with their controls and the mortality of the insects is counted daily. The process used for ovicidal testing is the same as that for adulticide testing. It consists of taking 12 peanut seeds, each carrying a single egg in place of the adults. Three repetitions are carried out, accompanied by their white witnesses. The boxes are placed on the benches until the first emergences and the latter are followed until the end when the seeds are crushed to obtain the number of dead larvae and pupae inside the seeds in order to be able to determine the number of hatched eggs as well as the unhatched ones as follows:

Number of eggs hatched = number of adults emerged + number of dead larvae + number of dead pupae.

Number of unhatched eggs = total number of eggs – number of eggs hatched.

Calculate the embryonic mortality rate using the following formula:

$$ME = \frac{\text{Number of unhatched eggs}}{\text{Total number of eggs}} \times 100$$

Fumigation of crushed fresh leaves

The fresh leaves are harvested early in the morning for reasons mentioned above and are then crushed using a blender. The crushed material is automatically placed in small jars (D = 10 cm, H = 5 cm) fitted with a mesh cover, which is then introduced into a large jar (H = 16 cm, D = 8 cm) containing adults, immediately closed hermetically with a non-mesh cover reinforced with adhesive tape to keep the volatile substances within the large jar. The same weights of leaves are used as before with three repetitions for each weight and a control without crushed leaves. Dead insects are also counted daily. The dead are recognized by any insect lying on its back and does not move its legs or antennae after agitation. The ovicidal tests are carried out in the same way as before. Peanut seeds, each carrying a single *C. serratus* egg, replace the adults in large jars.

Aqueous powder extract of dry leaves of *Carica papaya*

The papaya leaves harvested early in the morning are dried away from the sun on racks and ground using a blender. The powder is put in a jar in reserve for testing. We carried out a solid-liquid extraction whose solvent is rainwater; 200g of *C. papaya* leaf powder are extracted by maceration in 1L of water. The mixture is filtered using a filter, the extract is kept in a one-liter bottle for testing. Different concentrations (C1, C2 and C3) are made with dilution with rainwater using a micropipette. C1: Initial solution; C2: 2mL of C1 diluted with 2mL of rainwater and C3: 2mL of C1 diluted with 4mL of rainwater using a graduated micropipette.

To carry out the tests, the same boxes as those of the contact provided with white sheet are made available to us with the solutions already prepared. In each box we placed a piece of paper, using a micropipette 0.5 mL of prepared solution spread evenly on the paper. The adults are placed on the paper; three repetitions and two controls (blank control and solvent control) are carried out for each given extract concentration. After treatments, dead insects are counted daily in each box.

The mortality percentage is calculated per day as follows:

$$\% \text{ of Mortality} = \frac{\text{Number of dead adults}}{\textit{Total number of adults}} \times 100$$

For ovicidal tests, peanut seeds each carrying a single egg are sprayed by the dozen with 0.5mL of solution of each concentration using a micropipette.

In all calculations, the formula of Abbott is used to correct for natural mortality.

$$Mc = \frac{MT - MTo}{100 - MTo} \times 100$$

MTo = observed mortality, MT = control mortality and Mc = corrected mortality

The tables and graphs were produced in Excel 2013. The statistical analysis of the different parameters evaluated were carried out using R software version 3.2.4. This software allowed us to calculate the meaning, the standard deviation on the different repetitions of the mortality tests with the formulations and the comparison of the results for the different parameters studied.

3. Results

Impact of the three formulations based on *C. papaya* leaves on *C. serratus*

Contact with fresh crushed leaves of *C. papaya*

- On eggs

Contact with fresh crushed leaves of *C. papaya* reveals more or less significant mortalities disproportionate to the dose, between 41.85 and 81.18%. Thus, the average dose 15g induced the most mortality (81.18%) on *C. serratus* eggs while the lowest (10g) and the highest (20g) induced mortalities of 41.85% and 77.70% respectively. We note a significant difference between the mortalities induced by the 15g dose and the other doses (10g and 20g).

Table 1: Percentage of corrected egg mortality for contact tests with fresh crushed leaves of *Carica papaya*. The values are corrected means; those followed by the same superscript letter are not significantly different ($p \geq 0.05$).

Espèce	Doses (g)	Mortalité corrigée des œufs (%)
C. serratus	10	41,85a
	15	81,18b
	20	77,70b

- On adults

The analysis of Figure 1 shows that mortalities are proportional to the doses whatever the duration of treatment except on the first day when they are more or less significant with a higher percentage (69.92%) with the 15g dose. Maximum effectiveness (100% mortality) was observed from the 4th day with the highest dose (20g), on the 6th day with the medium dose. The 10g dose presents a constant of 88.33% from the 7th to the 19th day

before giving 100% mortality on the 20th day (end of treatment). Statistically, the mortalities induced by the three doses from the 1st to the 6th day show no significant difference $p \geq 0.05$, from the 6th day a significant difference is recorded ($p < 0.05$) between the first 5 and the last 15 days. The latter show no significant difference in mortality between them.

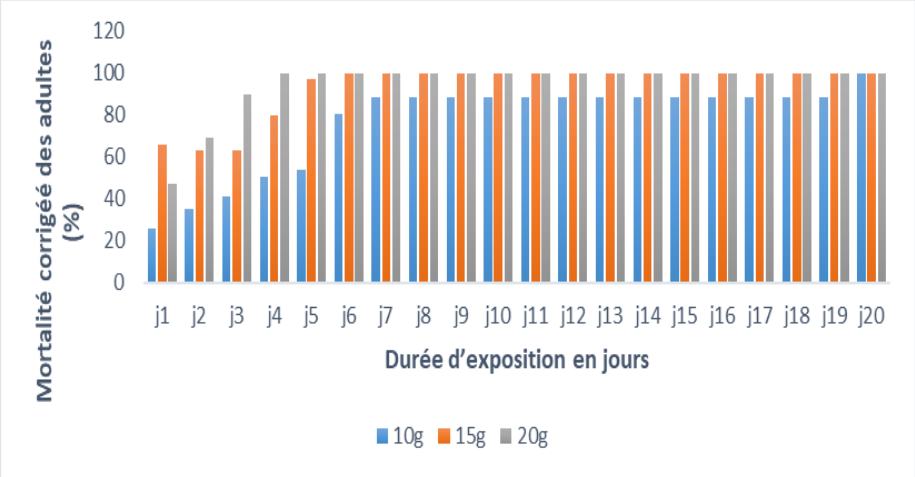

Figure 1: Percentage of corrected mortality of adults of Caryedon serratus induced by contact with crushed fresh leaves of Carica papaya

Fumigation of fresh crushed leaves of *C. papaya*

- On eggs

Fumigation of crushed fresh leaves induced equal mortalities of 67.61% on eggs treated with the application of the two lowest doses (10g and 15g). While the highest dose (20g) only promotes the survival of 2.81% of eggs (table 2).

Table 2: Percentage of corrected egg mortality for contact tests with fresh crushed leaves of *Carica papaya*. The values are corrected means, those followed by the same letter are not significantly different ($p \geq 0.05$).

Espèce	Doses (g)	Mortalité embryonnaire corrigée (%)
C. serratus	10	67,61a
	15	67,61a
	20	-2,81b

- On adults

We see in Figure 2 that on the first day of treatment the corrected mortalities are proportional to the doses applied with the highest dose which is well ahead at 75.72% over 8.41% (10g) and 17% (15g). On the 2nd day, the 20g dose retains its value but the lowest shoots up to 46.92% higher even than that of the second dose (40.82%). On the following two days, the lowest dose takes over and exceeds the highest with 79.59% on the 3rd and 73.53 on the 4th day. The 20g dose reverses this trend with 80.28% mortality on the 5th day and 74.07% on the 6th. A tie is noted between the two highest doses (68.47%) on the 7th day. From the 8th day the maximum score begins between the lowest and the highest dose at 82.10% before the second dose joins them on the 9th day where the progression of mortalities stops and stabilizes until the end of treatment. The statistical analysis shows no significant difference between the doses but significant differences in mortality exist for the two lowest doses during treatment.

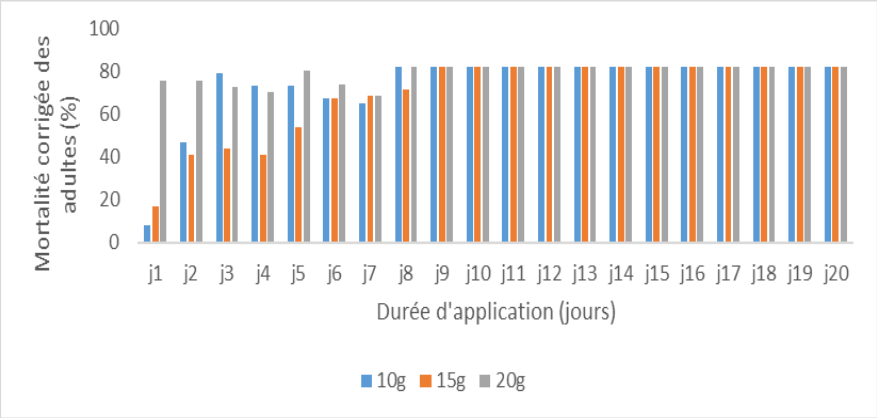

Figure 2: Percentage of corrected adult mortality of Caryedon serratus induced by fumigation of crushed fresh leaves of C. papaya

Aqueous extract of dried leaf powder of *C. papaya*

- On eggs

Table 3 reveals greater effectiveness of the C1 concentration on *C. serratus* eggs with 51.08% mortality. The other concentrations, C2 and C3 show an equal efficiency of 23.25%. Only the highest concentration gave mortalities greater than 50%. We do not observe any significant difference between the mortalities induced by the three concentrations.

Table 3: Percentage of corrected mortality of *Caryedon serratus* eggs induced by the aqueous extract of *Carica papaya* leaf powder. Values followed by the same alphabetical letter are statistically equal.

Espèce	Concentration	Mortalité embryonnaire corrigée
C. serratus	C1	51,08a
	C2	23,25a
	C3	23,25a

- On adults

Figure 3 highlights an effectiveness proportional to the concentration of the aqueous extract of *C. papaya* leaf powder on *C. serratus* adults. Thus, from the beginning to the end of treatment, the C1 concentration gave the greatest mortality. The 100% mortality is observable only with this concentration (C1) and from the 2nd day of application. The C2 concentration gives its greatest mortality from the eighth day (97.82%). The lowest dose (C3) follows this trend but gives this same value from the seventh day of treatment. Statistical analysis shows no significant difference between the mortalities induced by the different doses.

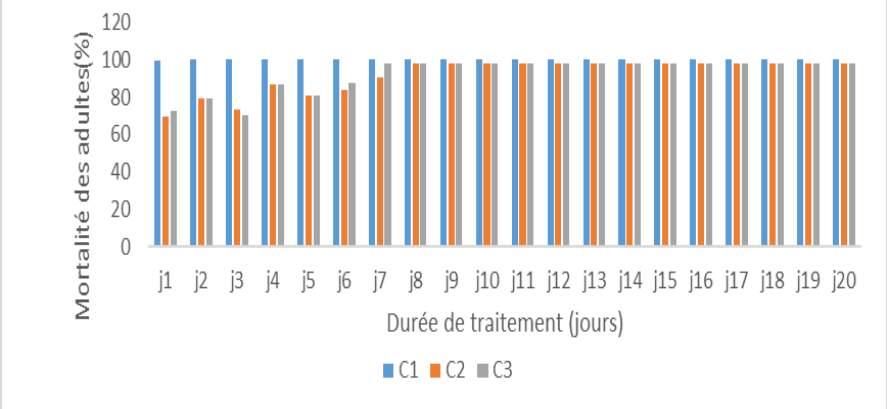

Figure 3: Percentage of corrected mortality of adults of Caryedon serratus induced by the aqueous extract of Carica papaya leaf powder

4. Discussion

The three formulations of *Carica papaya* leaves were tested on adults and on eggs of *Caryedon serratus* in the Laboratory under ambient conditions. The results indicate that these formulations (contact of fresh crushed leaves, fumigation of fresh crushed leaves and the aqueous extract of dry leaf powder) induce differential efficiencies over time following the dose tested. This is how contact with fresh leaves induced variable mortality in adults. These mortalities are spread over time and depend on the doses applied; 88.33% (10g) on the 7th day, 100% for high doses (15g and 20g) from the 4th day) on *C. serratus*. These results confirm those of [7] who tell us that Bioart showed remarkable adulticidal effectiveness (between 83.33% and 100%) with the application of all doses on *C. serratus*. Thus, the corrected egg mortalities caused by this formulation are more or less disproportionate to the dose and are between 41.85 and 81.18%. These results are in line with those of [8] who showed an ovicidal effect of 73.33% to 100% with this formulation based on Crateava religiosa on C. maculatus.

Fumigation of crushed fresh leaves also gave adulticidal effects spread over time. We recorded a maximum effectiveness of 82.10% with all doses in different exposure periods of 8 days (10g and 20g) and 9 days for 15g. It thus turns out that fumigation of fresh crushed leaves of *Carica papaya* is very effective on adults of *C. serratus*. The results of [9] confirm this with a remarkable effect of this plant on aphids at all stages of okra. This formulation also gave variable embryonic mortalities depending on the dose used, the two lowest doses caused mortalities equal to 67.61% and the highest dose proved not effective (- 2.81%) on *C. serratus* eggs. Our results disagree with those of [4] who showed that plant extracts, such as papaya, *Carica papaya* (caricaceae), are more effective at low doses against termites. The aqueous extract of the powder of dry leaves of this plant induces an effectiveness proportional to the concentration applied to the adults of *C. serratus* also vary according to the duration of exposure (100% mortality with C1 from the 1st day, C2 and C3 with mortalities equal to 97.82% on the 8th (C2) and the 7th day (C3)). [10] and [11] all obtained 100% mortality on adults of *C. serratus* with methanolic extracts of Calotropis procera and Boscia senegalensis respectively at a concentration of 0.1g/l within 24 hours following application. The results of [8] are also consistent with ours, 87.25% in C1 ($0.2g/cm^3$) and C2 ($0.13g/cm^3$) and 74.87% in C3 ($0.1g/cm^3$) with the aqueous extract of C. religiosa leaf powder on adults of C. maculatus. At the egg stage, the concentration (C1) gave the maximum mortality at 51.08%. Several authors such as [12] using plants to combat stored food pests have shown the effectiveness of plants including *Carica papaya* which we used in our tests. Thus, we also admit that all formulations prepared with *Carica papaya* leaves are effective on certain pests [13]. The results of [14] proved this, saying whatever the leaf and the extraction method used, papaya remains effective for treating cowpea pests. [10] and [11] all obtained 100% mortality on adults of *C. serratus* with methanolic extracts of Calotropis procera and Boscia senegalensis respectively at a concentration of 0.1g/l within 24 hours following application. The results of [8] are also consistent with ours, 87.25% in C1 ($0.2g/cm^3$) and C2 ($0.13g/cm^3$) and 74.87% in C3 ($0.1g/cm^3$) with the aqueous extract of C. religiosa leaf powder on adults of C. maculatus. At the egg stage, the concentration (C1) gave the maximum mortality at 51.08%. Several authors such as [12] using plants to combat stored food pests have shown the effectiveness of plants including *Carica papaya* which we used in our tests. Thus, we also admit that all formulations prepared with *Carica papaya* leaves are effective on certain pests [13]. The results of [14] proved this, saying whatever the leaf and the extraction method used, papaya remains effective for treating cowpea pests.

5. Conclusion

This study states that *C. papaya* leaves have a biocidal impact on the pest *C. serratus*, which varies depending on the leaf formulation used (contact of crushed fresh leaves, fumigation of crushed fresh leaves and aqueous extract of dry leaf powder). Our results reveal differential efficiencies with the application of doses for each formulation. Contact with crushed fresh leaves is the most effective formulation on the eggs and adults of *C. serratus*. We plan to test the other parts of the plant for this pest. Furthermore, we also want to extend this study on crop protection with processes still applicable by farmers using native plants in order to highlight certain plant species which seemed useless, but in reality are of major importance in the fight against crops and stored foodstuffs.

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