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Research Article

BIOCIDAL IMPACT OF DIFFERENT FORMULATIONS BASED ON *CARICA PAPAYA* LEAVES ON THE EXTERNAL FORMS OF *CALLOSOBRUCHUS MACULATUS* (FABRICIUS, 1775), THE MAIN PEST OF COWPEA STOCKS IN SENEGAL

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ABSTRACT

Papaya leaves (*Carica papaya*) were tested on eggs and adults of the pest *Callosobruchus maculatus*. Different formulations based on this plant have been applied to these forms of the pest. The results noted high adult mortality varying depending on the doses, duration of exposure and the formulation applied. The crushing of fresh leaves caused considerable mortality in adults through contact. These are spread over time depending on the doses applied, 55.75% (10g) on the 5th day, 100% for high doses (15g and 20g) from the 10th day. The corrected mortalities induced by this formulation on eggs are very high and proportional to the dose (10g, 15g and 20g gave respectively 93.63%, 97.08% and 100% mortalities). Fumigation of fresh crushed leaves also proved effective on adults with mortalities disproportionate to the dose of between 57.97% and 97.82% around the 3rd and 4th day with the application of all doses. It also gave an embryonic mortality of 94.70% whatever the dose applied. The aqueous extract of the powder of dry leaves of this plant (*Carica papaya*) showed a variation of the effects over time with the application of different concentrations, the highest mortality 22.93% was recorded with concentration C2 on the 2nd day while C1 and C2 induced respective mortalities of 11.47% and 20.02%. At the egg stage, the aqueous extract induced average mortalities disproportionate to the dose with greater mortality in C2 (72.72%).

Keywords: *Carica papaya, Callosobruchus maculatus*, Fumigation, Contact, Powder, Aqueous extract, Fresh leaves.

INTRODUCTION

Callosobruchus maculatus is one of the insects that exert permanent pressure on cowpea at the post-harvest stage (Brice *et al.*, 2016). The damage observed on cowpea is caused by the juvenile stages of the insect. Before the pupal stage, a significant part of the nutrient reserves of the cowpea seed cotyledons is consumed. The activity of consuming seed reserves by the larvae causes heat and humidity which leads to the development of fungi such as *Aspergillus sp*., *Fasarium sp.*, *Botrytus sp*. (Adebayo *et al.*, 2019) leading to quantitative losses. In rural areas where conservation techniques for agricultural products are poorly developed, this pest causes post-harvest losses of up to 100% in a few months (Lienard *et al.*, 1994). The cowpea weevil, *C. maculatus*, not only causes a reduction in dry weight, but also a reduction in seed quality and seed viability, compromising their consumption and germination. In some villages, damage can even reach 100%. It is the most formidable insect that destroys cowpea seeds and can remain in stocks during storage.

The infestation which had also started in the field continues in stocks on seeds and dry pods. This causes enormous damage and consequently losses ranging from 80 to 100% after 5 to 6 months of storage (Thiaw, 2008). 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 handling and non-compliance with waiting periods are responsible for the resistance of certain harmful strains, the elimination of natural enemies, health problems (consumers and farmers)

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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). However, with the intensification of agriculture and restrictions on the use of pesticides due to their toxic effects, we wanted to promote local knowledge of plants with pesticidal properties.

MATERIAL AND METHODS

The strain of *C. maculatus* used in the experiment comes from mass breeding carried out at the entomology and acarology laboratory of the Department of Animal Biology (FST/UCAD) on cowpea seeds. The plant organs used are cowpea seeds and papaya leaves. The cowpea seeds served as a food support for the insects; they 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 at the Botanical Garden of the Department of Plant Biology (FST /UCAD/Senegal).

Breeding was necessary to obtain a large number of individuals available for testing. Specimens of *C. maculatus* are introduced into perforated plastic boxes 6.5 cm in diameter and 4.5 cm in height containing cowpea 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 tests or to maintain mass breeding. The sexes are identified as follows: the male has a small pygidium covered by the elytra while the female has an enlarged pygidium not covered by the elytra.

Procedure for contacting fresh crushed leaves of *Carica papaya* on adults and eggs of *C. maculatus*

The *Carica papaya* leaves used for contact tests are harvested early in the morning before sunrise to optimize the concentration of active substances. 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. maculatus* numbering 12 individuals per box. All tests are repeated three times with their controls. Insect mortality is counted daily. The process used for ovicidal testing is the same as that for adulticide testing. It consists of taking 12 cowpea seeds, each carrying a single egg in place of the adults. Three repetitions are carried out, accompanied by their witnesses. The boxes are placed on the benches until the first emergences. These boxes are followed until the end of the experiment and the seeds are then crushed to see the number of dead larvae and pupae inside the seeds. This allowed us to determine the number of hatched eggs and that of unhatched eggs as well as the embryonic mortality rate using the following formulas:

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;

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

Procedure for fumigating fresh crushed leaves of *Carica papaya* on adults and eggs of *C. maculatus*

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 not making movements of its legs and antennae after agitation. The ovicidal tests are carried out in the same way as before. Cowpea seeds, each carrying a single *C. maculatus* egg, replace the adults in the large jars.

Procedure of aqueous extract of powdered dry leaves of *Carica papaya* on adults and eggs of *C. maculatus*

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 filter paper and the filtrate 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 is the initial solution, then C2 and C3 are obtained by dilution C1 with rainwater: 2mL of C1 diluted with 2mL of rainwater; C3: 2ml of C1 diluted with 4mL of rainwater using a micropipette.

To carry out the tests, the boxes used are the same as those of the contact, but they are provided with white sheet thus facilitating the movement of the insects in the jars. In each 0.5 mL of prepared solution is spread evenly on the paper using a micropipette. 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:

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

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

In all calculations, the formula of Abbott *et al.*, (1925) was used to correct for natural mortality observed in controls.

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

Mc = corrected mortality; MTo = observed mortality; MT = control mortality

Fertility of females from treated eggs

Of all the ovicidal tests carried out with the three formulations, only *C. maculatus* females emerging from eggs treated with the aqueous extract are monitored for this parameter. A male and a female from the treated eggs are paired for 10 days on healthy cowpea seeds in order to count the number of eggs laid per female using a monocular magnifying glass. Fertility is assessed by counting the eggs laid by females from treated eggs.

Sex ratio (R) of the first offspring (F1) of adults from eggs treated with *C. maculatus* with the aqueous extract

It is determined by the percentage ratio of females to all offspring. If it is greater than 50%, the sex ratio favors females, otherwise it is in favor of males. The tables and graphs were produced in Excel 2013. The statistical analyzes of the different parameters evaluated were carried out using R software version 3.2.4. This software allowed us to calculate the mean, 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.

RESULTS AND DISCUSSION

Figure 1 shows ineffectiveness (2.75%) of the second dose (15g) on the first day when the lowest dose gives a mortality of 5.5% and the highest dose 14.17% mortality. This trend is reversed in the following 9 days where the three doses induce almost the same mortality in adults. The dose (15g) shows overall greater effectiveness on adults with 100% corrected mortality from the tenth day of application while the highest dose showed 100% on the fourteenth day and the lowest showed 55.75% mortality on the fifth day. The statistical analysis shows that the three doses induced the same effects on adults during treatment while there are significant differences within a given dose depending on the duration of application (Figure 1).

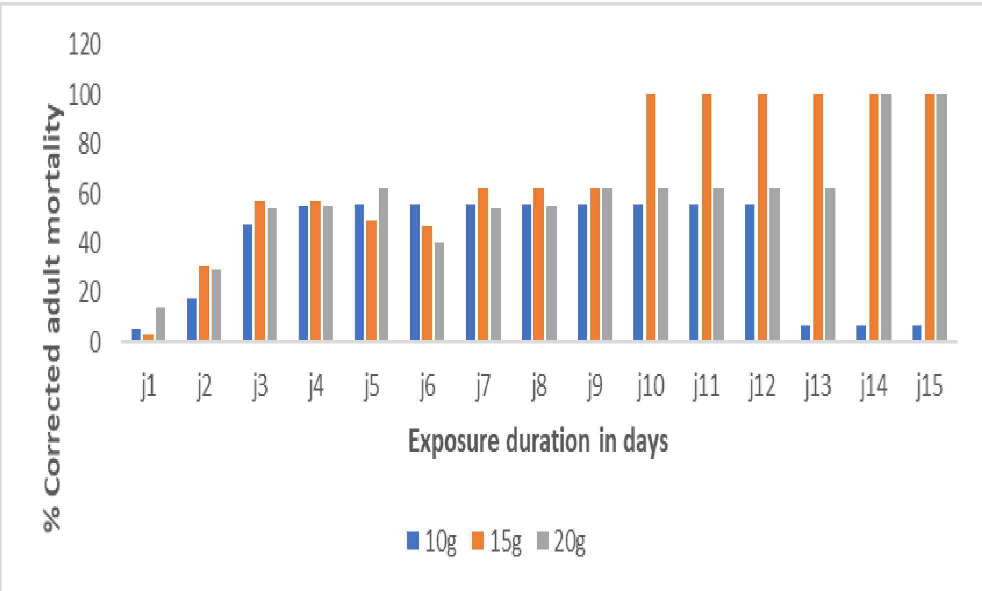

Figure 1. Percentage of corrected mortality of adults of *Callosobruchus maculatus* induced by contact with crushed fresh leaves of *Carica papaya*.

Contact with crushed fresh leaves of *C. papaya* on *C. maculatus* eggs revealed high mortalities proportional to the applied dose, ranging from 93.63 to 100%. Thus, the highest dose, 20g, induces 100% mortality while the lowest dose (10g) and the medium dose (15g) induce respective mortalities of 93.63% and 97.08% (Table 1).

Table 1. Corrected mortality percentage of *C. maculatus* eggs for contact tests with crushed fresh leaves of *Carica papaya*. The values are corrected means, those followed by the same letter are not significantly different ($p \geq 0.05$).

Species	Doses (g)	Corrected embryonic mortality (%)
C. maculatus	10	93,63[a]
	15	97,08[a]
	20	100[a]

The analysis in Figure 2 shows a higher effectiveness of the lowest dose until the tenth day when they give the same mortality 6.34%. On the 1st and 2nd day the trend gives an inversion of the proportionality of the doses for induced mortality (the lower the dose, the more it causes adult mortality). This trend is reversed in the following 7 days where the highest dose takes second place. The 15g dose gives no mortality on the 8th and 9th day. From the 11th day the treatment remains ineffective for all doses applied until the end of monitoring. Each of the doses draws its maximum mortality on a given day, 10g and 20g respectively give (97.82%) (73.80%) on the 3rd day and the 15g dose gives its highest mortality on the 4th day with 57.97%. Statistically significant differences in mortality are recorded between the three doses but no significant difference is noted for the same dose throughout the treatment (Figure 2).

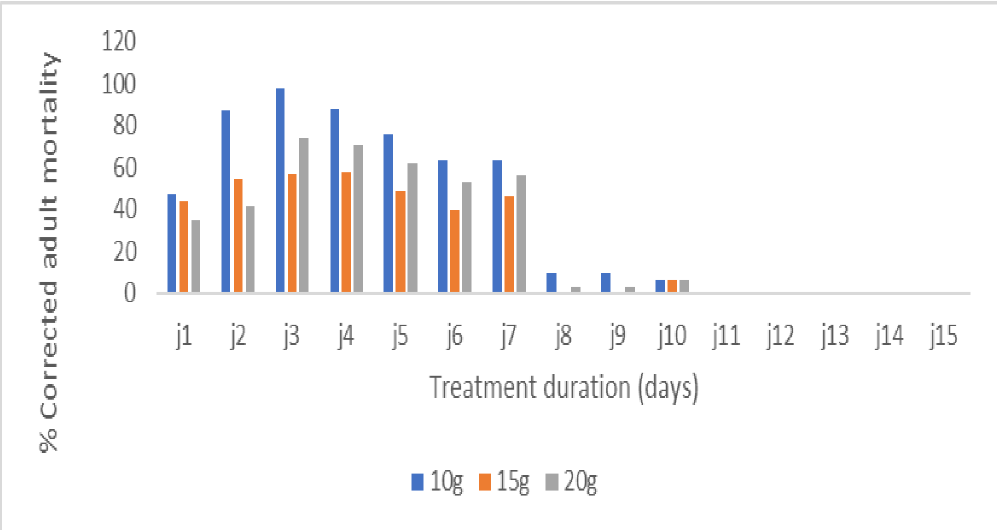

Figure 2. Percentage of corrected adult mortality of *Callosobruchus maculatus* induced by fumigation of crushed fresh leaves of *Carica papaya*.

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 ($p \geq 0.05$).

Species	Doses (g)	Corrected embryonic mortality (%)
C. maculatus	10	94,70[a]
	15	94,70[a]
	20	94,70[a]

Fumigation of crushed fresh leaves induced a maximum mortality of 94.70% on eggs treated with the application of all doses (Table 1). Figure 3 shows negative effects that begin on the sixth day of treatment. In the first 5 days no negative effects were recorded but rather greater effectiveness of the second dose (C2) on adults of *C. maculatus*. This dose gave the highest mortality on adults on the second day with 22.93% and causes a resistance of -15.02% starting from the thirteenth to the fifteenth day of application. Concentration C1 causes its greatest mortality on the fourth day with 11.47% and strong resistance on the thirteenth day of treatment (-23.75%). The C3 concentration is the most effective in terms of mortality (20.02%) on the second day, with a resistance of -35.09% of adults. The treatment experiences a variation in effects over time with the application of different concentrations. This is how the negative effects of the highest and lowest doses (C1 and C3) begin on the 6th day while those of C2 begin on the 13th day. This formulation does not induce any significant difference in mortality whatever the dose applied to adults of *Callosobruchus maculatus* (Figure 3).

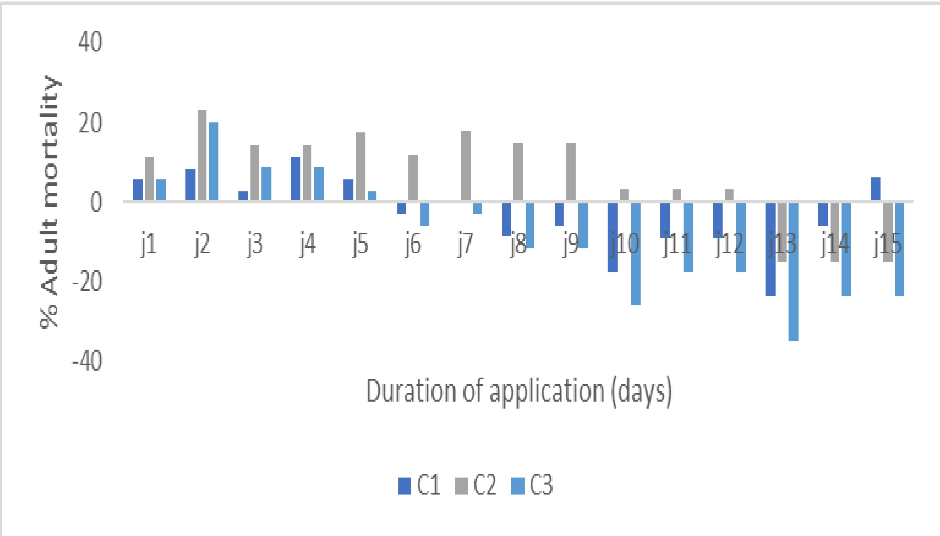

Figure 3. Percentage of corrected adult mortality of *Callosobruchus maculatus* induced by the aqueous extract of *Carica papaya* leaf powder.

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

Species	Concentration	Corrected embryonic mortality
C. maculatus	C1	62,95^{a}
	C2	72,72^{a}
	C3	22,94^{b}

Table 3 reveals greater effectiveness of the C2 concentration on *C. maculatus* eggs with 72.72% mortality. The other concentrations, C1 and C3 respectively show mortalities of 62.95% and 22.94%. We observe a significant difference between the mortalities induced by the two highest concentrations (C1 and C2), but differ significantly from those produced by the lowest concentration C3. Of all the adults emerging from *C. maculatus* eggs treated with the three formulations and their different doses, there was only one possibility of continuing female fertility: females of *C. maculatus* (aqueous extract). That is to say on all the others, there was a low rate of emergence or a total absence of pairs emerging on the same day (for a given dose). Hence the inability to make couplings, to continue the fertility of females and finally to determine the Sex ratio of the first offspring (F1) from these survivors. Figure 4 represents the fecundity of females of *C. maculatus* from eggs treated with the aqueous extract of *C. payaya* leaf powder with the three concentrations followed by the controls (White and Solvent).

Observation of the results on the average of eggs laid per female shows that the aqueous extract of *C. papaya* leaf powder reduces the fecundity of females from treated eggs compared to the white control in a manner proportional to the concentration tested. We record for the highest concentration; a rescued female gives on average 26.33 eggs. With the two other concentrations (C2 and C3) a female gives an average of 40.67 and 42.33 eggs respectively, while the females laid 93 eggs for the white control and 80.33 eggs for the solvent control. For all the concentrations tested and their controls, the number of eggs laid sharing the same superscript letter, show no significant difference between them ($p > 0.05$) (Figure 4).

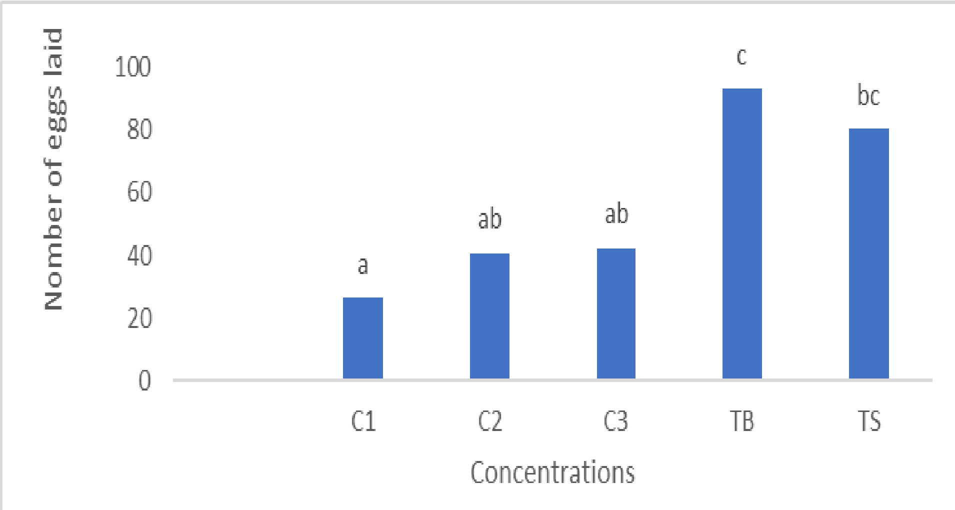

Figure 4. Fecundity of *C. maculatus* females from eggs treated with the aqueous extract of *C. papaya* leaf powder.

The analysis of Figure 5 highlights the effect of the aqueous extract of *C. papaya* leaf powder on the nature of the sex of the first offspring (F1) of the adult *C. maculatus* survivors emerging from the ovicidal tests with the application of the three concentrations accompanied by their controls (White and Solvent). It turns out that the Sex ratio is in favor of males for all the concentrations tested unlike the controls for which it is in favor of females with percentages of 60.33% (TB) and 61.33% (TS). We notice that this favor attenuates with the highest and lowest concentration (C1 and C3). This is how C2 gives a percentage of 31.67% while the others give respectively 36% (C1) and 40.67% (C3) Sex ratio. Statistically, the product shows no significant difference in sex ratio between the doses applied ($p > 0.05$) (Figure 5).

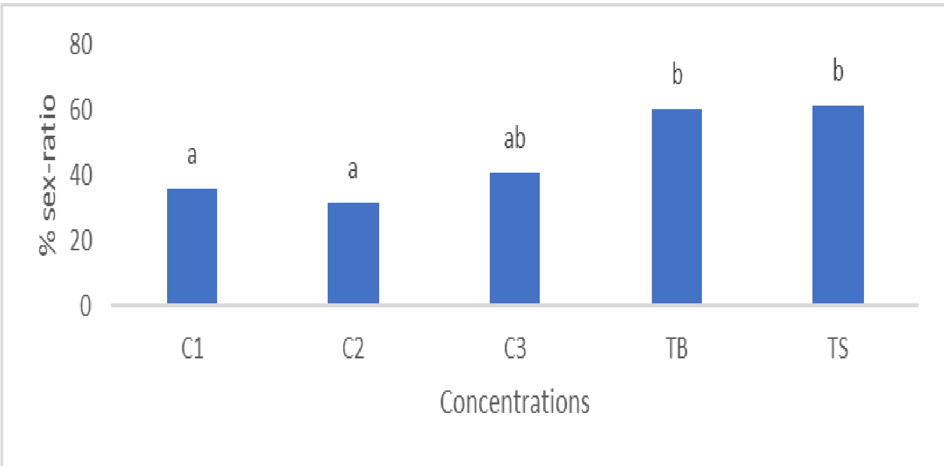

Figure 5. Sex ratio of the first offspring (F1) of *C. maculatus* adults from eggs treated with the aqueous extract of *C. papaya* leaf powder.

The three formulations of *Carica papaya* leaves were tested on adults and on eggs of *Callosobruchus maculatus* in the Laboratory under ambient conditions. The results indicate that these formulations (contact with 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; 55.75% (10g) on the fifth day, 100% from the tenth day with the average dose (15g). These results are in agreement with those of Fall *et al.* (2024) 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 very high and proportional to the dose, between 93.63% and 100%. These results are in line with those of Faye (2015) who showed an ovicidal effect of 73.33% to 100% with this formulation based on *C. religiosa* on the same species. Fumigation of crushed fresh leaves also gave adulticidal effects spread over time. We recorded different mortalities, the 10g and 20g doses respectively gave 97.82% and 73.80% on the third day, while the 15g dose induced 57.97% mortality on the fourth day. It thus turns out that fumigation of fresh crushed leaves of *Carica papaya* is effective on adults of *C. maculatus*. The results of Gnago (2010) confirm this with a remarkable effect of this plant on aphids at all stages of okra. This formulation also gave an effectiveness of 94.70% regardless of the dose applied. For the aqueous extract of the powder of dry leaves of this plant (*Carica papaya*), a variation of the effects over time was noted with the application of different concentrations, the highest mortality 22.93% was recorded with concentration C2 on the 2nd day while C1 and C2 induced respective mortalities of 11.47% and 20.02%. Consequently, our results disagree with those of Faye (2015) who obtained an effectiveness of 87.25% in C1 (0.2g/cm3) and in C2 (0.13g/cm3) and 74.87% in C3 (0.1g/cm3) with this formulation based on *C. religiosa* on adults of the same species. This could be explained by the composition of the plant or variable temperature conditions. At the egg stage, the aqueous extract induces average mortalities disproportionate to the dose with greater mortality in C2 (72.72%). Our results corroborate those of Pérez-Gutiérrez *et al.* (2011), who observed a 48.5% to 58.3% reduction in the number of viable larvae of *Spodoptera frugiperda* by the application of chloroform extract of *Carica papaya*.

According to Tahiri *et al.* (2010), the effect of the alcoholic extract of the seeds contained in the green fruit and that of the hexanic extracts of the pulp and ripe seeds of *C. papaya* are toxic towards *Macrotermes bellicosus* termites. These three extracts induce similar lethal doses (LD50) which range respectively from 0.15 ± 0.0 mg/L, 0.16 ± 0.0 mg/L and 1.06 ± 0.0 mg/L. Several authors using plants to fight pests have shown the effectiveness of plants including *Carica papaya* which we used in our tests. Thus, for all stages, it is clear that the nature of the product (solid, gas, liquid) plays a preponderant role in all the results obtained. Kandji made the same remark in 1996 with his results which show us that the nature of the product can change its activity on adults of *C. serratus*. Observation of the results on the average of eggs laid per female shows that the aqueous extract of *C. papaya* leaf powder reduces the fecundity of females from treated eggs compared to the white control in a manner proportional to the concentration tested. Our results corroborate with those of Thiaw *et al.* (2015), who found that biocidal extracts of *S. occidentalis* reduce the fecundity of females from eggs treated by 27.29% with the methanolic extract and by 67.2% with the ethyl acetate fraction on *C. serratus*. Kongne *et al.*, recorded mortalities of 73.33 ± 5.77 in *C. maculatus* with the application of the methanolic extract of *C. papaya* powder. It can be said that the effect of the aqueous extract of papaya leaf powder is not only fatal but can also affect the fertility of females hatched from the treated eggs. This remark is confirmed by Seck (1994) whose leaf powders of *Ocimum sanctum* and *Curcuma longa*, which he used, have a harmful effect on the fertility of females of *C. maculatus*. Adenekan *et al.*, 2019 showed the toxicity of different parts of *Carica papaya* (leaves, flower, seeds) against *Sitophilus zeamais*. In the same vein, Medona *et al.* (2025) showed repellent effects of 98.4% and insecticidal effects of 89.5% of *Cymbopogon citratis* against *Sitophilus granarius* in cereal stocks. This reduction could be explained by the short lifespan of females due to the biocidal effect of the product. Thus the results obtained with the aqueous extract of *C. papaya* on the nature of the sex of the first offspring (F1) of adults of *C. maculatus* surviving the ovicidal tests showed that the Sex ratio is in favor of males for all concentrations tested unlike those of the controls for which it is in favor of females with percentages of 60.33% for the White Control and 61.33% for the Control Solvent. These results do not go in the same direction as those of Gningue *et al.* (2019), for which the sex ratio was in favor of females with the application of *A. indica* on *C. serratus*. It can be concluded that the predominance of males reduces the risk of population increase in stored seeds, hence the prevention of damage in storage areas.

CONCLUSION

This study states that *C. papaya* leaves have a biocidal impact on the pest *C. maculatus*. This effect varies depending on the leaf formulation used (contact of fresh crushed leaves, fumigation of fresh crushed leaves and aqueous extract of dry leaf powder). Our results reveal differential efficiencies with the application of doses for each formulation. The two formulations of fresh leaves (contact and fumigation) are the most effective formulations on adults and eggs while the aqueous extract of dry leaf powder remains the least effective whatever the stage of development of the insect. We thus highlighted the insecticidal effect of *C. papaya* on *C. maculatus*. 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 pests of crops and stored foodstuffs.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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