

# Efficacy of Neem and Papaya Leaf Powders on the Cowpea Bruchid *Callosobruchus maculatus* (Coleoptera: Chrysomelidae)

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## ABSTRACT

*Callosobruchus maculatus* Fab (Coleoptera: Chrysomelidae) is a pest that causes enormous damage to cowpea stocks. To limit this damage, farmers resort to synthetic insecticides despite their consequences on the environment and human health. The objective of this study was to evaluate the efficacy of neem and papaya leaf powders as an alternative to control *C. maculatus* pests of *Vigna unguiculata* Walp. stocks (Fabaceae). Five hundred grams (500 g) of cowpea seeds contained in one-litre glass jars were infested with 10 pairs of *C. maculatus* aged 24 hours. Each of the powders was used to treat the seeds at 20, 40, 60 g/kg cowpea in each jar respectively. For each dose of each biopesticide used, four batches (Batch 1, Batch 2, Batch 3 and Batch 4) were prepared and monitored after 1, 2, 3 and 4 months of storage respectively. The rate of reduction of the *C. maculatus* population, and the weight losses of the cowpeas, were determined. The results showed that after 4 months of cowpea storage, neem caused, at doses of 20, 40 and 60 g/kg, a significant reduction in the bruchid population by  $42.58 \pm 6.97\%$ ;  $79.1 \pm 5.31\%$  and  $84.27 \pm 5.7\%$ ; while at the same doses, papaya reduced the population by  $14.32 \pm 4.53\%$ ;  $42.18 \pm 5.83\%$  and  $64.86 \pm 8.03\%$  compared to the negative control. No bruchids emerged from seeds treated with the synthetic insecticide (BEXTOXIN: fumigant) used at the dose of 0.25 g/kg during all storage periods. All doses significantly reduced cowpea weight losses, especially with the higher doses. Neem leaf powder was more effective than papaya leaf powder. Neem leaf powder could therefore be used as an alternative to synthetic chemicals in pest management of cowpea stocks.

**Keywords:** *Callosobruchus maculatus*, Neem, Papaya, Togo, *Vigna unguiculata*.

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## I. INTRODUCTION

Cowpea (*Vigna unguiculata* Walp.) is a thermophilic tropical herbaceous plant as it thrives in conditions of intense heat and light. It occupies an important place in the diet and is the most important seed legume cultivated in tropical Africa because of its energy value and high protein content [1]. Cowpea is an important source of protein, which gives it the name "poor man's meat" [2]. In addition to its nutritional value, cowpea has a definite economic value. Sub-Saharan Africa provides about 95% of the world's cowpea production, with more than 80% of Africa's share being produced in West Africa. Nigeria is the world's largest cowpea producer (and consumer) followed by Niger and Burkina Faso [3].

The increase in area planted and the cultivation of high-yielding cowpea varieties means that there is more seed to store. However, there are conservation problems. Stored legumes suffer enormous damage by a variety of pests. Destructive agents of stored cowpeas are insects, microorganisms and rodents. The insects that attack cowpea seeds are mainly bruchids. In the humid zone of Togo, three species of cowpea crops and stocks occur *Callosobruchus maculatus* (F.), *Bruchidius atrolineatus* (Pic.) and *Callosobruchus rhodesianus* (Pic.) [4]. Among the cowpea

predatory bruchids, *C. maculatus* (F.) is the most damaging. This insect can cause 100% crop loss in three months in sub-Saharan Africa [5]-[6]. Damage caused by these beetles on stored cowpea seeds for consumption or cultivation includes, among others, physical deterioration of seeds, loss of organoleptic characteristics and loss of germination capacity [7]. Given the seriousness of post-harvest losses caused by insects, Labeyrie [8] said that in Africa, farmers work for the insects. It should be noted that infestation starts in the field and continues during storage [5]-[9]. The bruchid, *C. maculatus* feeds on cowpea seeds by perforating round holes in them and consuming the nutrient reserves contained in the cotyledons, causing quantitative and qualitative losses [10].

Various control methods are used to deal with these post-harvest losses. These include chemical control, biological control, the use of plant biocides, physical methods, and varietal resistance [1]. According to PAN Africa [11], synthetic chemical insecticides are abused and misused in most African countries. However, these synthetic pesticides, although effective, do not only cause resistance problems in insect pests and destroy beneficial organisms, but also lead to harmful effects on the environment and human health [12]. Alternative control methods that are cheap and effective and respect the environment and human health are advocated.

Many local plants appear to be effective in controlling insects in stored commodities. The use of plants as biopesticides can significantly reduce pest pressure and the need for synthetic pesticides [13]. Pesticides based on locally available plant extracts are of great interest [7]-[14]. Neem is one of the plants that have been the subject of a large number of scientific studies on natural pesticides [15]. Gauvin et al. [16] found no relationship between the concentration of azadirachtin and the insecticidal effect of the neem extracts tested. These authors suggested the importance of evaluating the total insecticidal effect of neem extracts and not only their azadirachtin concentration.

Papaya leaves contain papain as active substances that act on crop pests [17]. Neem and papaya leaves have been shown to be effective in pest management [15]-[18]. These leaves could be an alternative for the conservation of stored commodities such as cowpea in storage against bruchids.

The general objective of our study is to contribute to the reduction of cowpea stock losses caused by *C. maculatus* attacks through the use of plant-based substances as an alternative control method. Specifically, it is to determine the effect of neem and papaya leaf powders on the population size of *C. maculatus* in stocks; to evaluate the effect of these powders on bruchid damage reduction during storage; and to determine the effectiveness of the treatments used for the conservation of cowpeas.

## II. MATERIALS AND METHODS

### A. Material

#### 1) Plant material

##### 1.1) Cowpea: *Vigna unguiculata* (L.) Walp

The cowpea used was the variety "VITOCO" bought at "Gléi" market in Ogou prefecture (Togo). Perforated, mouldy, broken seeds and impurities (sand, plant debris) were removed from the stock. Healthy (sorted) cowpea seeds were then put in a freezer for two (2) weeks to eliminate any viable organisms from them. Two days before use in the experiment, the seeds were taken out of the freezer to defrost them. They were then dried in a room at breeding temperature.

##### 1.2) Preparation of biopesticide powders

Neem and pawpaw leaves were collected in March 2020 from the University of Lomé (Togo). The leaves were dried in an air conditioned room and then reduced to fine powders in a wooden mortar and sieved with a 0.2 mm mesh size sieve to obtain a fine powder. The resulting green leaf powders were then used to preserve cowpea stocks.

#### 2) Insects rearing

The *C. maculatus* bruchid used in this study was reared from individuals emerging from cowpea initially purchased at the 'Gléi' market. For rearing, approximately 200 adult *C. maculatus* individuals of undetermined sex were introduced into separate Plexiglas boxes containing 500 g of cowpea. After 24 hours, these cultures were sieved with a 3 mm mesh size sieve to separate the adults from the substrate. The first emergent insects were recovered after 22 days of incubation. Individuals from this generation were used in our tests.

#### 3) Chemical insecticide

The chemical insecticide used as a positive control was BEXTOXIN (Aluminium Phosphide 57.0%) purchased from a phytosanitary store in Lomé (Togo). It is a fumigant often used by farmers and traders against stock insects.

### B. Methods

#### 1) Experimental set-up

The tests were carried out at the Laboratory of Ecology and Ecotoxicology of the University of Lomé (Togo). Three (3) doses (D1 (20 g/kg cowpea), D2 (40 g/kg cowpea), D3 (60 g/kg cowpea) of neem and papaya leaf powders were tested. Five hundred grams (500 g) of cowpea seeds contained in 1-litre glass jars were infested with ten (10) pairs of *C. maculatus* aged 24 hours. One week after infestation, the adults were removed. Four batches (batch 1, batch 2, batch 3 and batch 4) were formed. These batches were treated with neem leaf powder at dose D1 (20 g/kg). As soon as they were treated, the jars were closed with perforated lids to ensure gas exchange and then covered with a fine cloth to prevent the exit of bruchids. For each batch four replicates were made. Batch 1, batch 2, batch 3 and batch 4 were examined and destroyed after one, two, three and four months of storage respectively. The same set-up was used for the other treatments, namely neem D2 (40 g/kg cowpea), neem D3 (60 g/kg cowpea), papaya D1 (20 g/kg cowpea), papaya D2 (40 g/kg cowpea), papaya D3 (60 g/kg cowpea). The jar treated with BEXTOXIN (insecticide) and the untreated jar (control 1) served as reference and negative control respectively. Another positive control (control 2) was set up containing 60 g/kg cowpea seed powder. For these three (3) treatments, the same set-up was used.

#### 2) Dose of synthetic insecticide used

0.125 g of BEXTOXIN (fumigant) corresponding to a dose of 0.25 g/kg was used as our positive control. This mass was obtained by dividing a 3-g tablet into 24 parts. The product was tied up in a piece of cloth before putting in the jars.

#### 3) Effect of treatments on the rate of reduction in *C. maculatus* population

The four jars corresponding to the same dose of powder were examined after 1, 2, 3 and 4 months of storage. The numbers of live and dead adult *C. maculatus* were determined. The counting procedure consisted of tipping all the contents of a box into a canvas counting a cage or a small net bag of 1 mm mesh size to prevent the live adults from flying away. Using an insect suction device and flexible forceps, live and dead bruchids were separated from the cowpea and counted.

The rate of population reduction was determined relative to the negative control (control 1) using the following formula:

$$R (\%) = [100 - (Be / Bt \times 100)]$$

where Be = number of bruchids in the trial. Bt = number of bruchids in the negative control.

#### 4) Assessment of treatment efficacy

Efficacy was determined by the Weevil Perforation Index (WPI). According to Fatope and Nuhu [19], it is calculated in the case of treated stocks using the following formula:

$$\text{WPI (\%)} = \text{Pe} / (\text{Pt} + \text{Pe}) \times 100$$

where Pe = rate of attacked seeds in the trial. Pt = rate of attacked seeds in control 1

If WPI is less than 50%, then the treatment is effective.

If WPI is higher than 50%, then the treatment is ineffective.

### 5) Effect of treatments on reducing cowpea seed mass loss

The mass of the seeds in each jar was determined at the end of storage and mass loss was evaluated by the formula:

$$\text{Mass loss} = \text{initial mass} - \text{final mass}$$

### C. Statistical Analysis

The results obtained were statistically analysed using GenStat edition 12 software. The data were subjected to an analysis of variance (ANOVA). The separation of the means was done by applying the Student-Newman-Keuls test at the 5% threshold.

## III. RESULTS

### A. Effect of Treatments on the Rate of Reduction of *C. maculatus* Adults

The average rate of reduction of *C. maculatus* adults with the synthetic insecticide was 100% (Fig. 1).

This rate was significantly higher than the rates recorded in the other treatments during the entire conservation period. By day 30 of storage, the biopesticides used had no effect on bruchid population reduction. The average rates of reduction in adult *C. maculatus* in control 1 (0%), control 2 (0%), neem D1 (3.78±1.79%), neem D2 (13.87±4.57%), neem D3 (13.46±3.93%), papaya D1 (4±1.4%), papaya D2 (8.46±2.19%) and papaya D3 (7.52±3.88%) were not significantly different ( $P = 0.979$ ).

At day 60, the mean rates of reduction in adult *C. maculatus* in neem D1 (65.75±8.86%), neem D2 (82.57±9.04%), neem D3 (83.32±7.75%), papaya D2 (65.25±11.8%) and papaya D3 (82.37±10.45%) treatments were statistically identical to each other ( $P = 0.332$ ), but higher than the results for papaya D1 (25.64±5.62%), control 1 (0%) and control 2 (0%) which were significantly equivalent to each other (Fig. 1).

At day 90, the mean reduction rates of *C. maculatus* adults in the neem D2 (89.75±0.98%) and neem D3 (92.02±0.61%) treatments were statistically identical, but higher ( $P < 0.001$ ) than the results of the papaya D2 (71.86±3.09%) and papaya D3 (82.98±4.71%) treatments. The mean reduction rates of the treatments and papaya D3 were not different, but statistically higher ( $P < 0.001$ ) than the rate obtained in the neem D1 treatment (66.65±7.1%). In the neem D1 treatment the result was higher than the result in the papaya D1 treatment (14.32±4.53%). The average reduction rate obtained in the papaya D1 treatment was significantly higher ( $P < 0.001$ ) than the rates obtained in control 1 (4225 sprays) and control 2 (0%).

At 120 days of storage, the mean reduction rates of *C. maculatus* adults in neem D2 (79.1 ± 5.31%) and neem D3 (84.27 ± 5.7%) treatments were identical, but significantly higher ( $P < 0.001$ ) than the rate obtained in papaya D3

(64.86±8.03%). However, this average rate was statistically higher ( $P < 0.001$ ) than those recorded in the papaya D2 (42.18 ± 5.83%) and neem D1 (42.58 ± 6.97%) treatments. In the neem D1 and papaya D2 treatments, the average reduction rates were statistically identical to each other, but significantly higher ( $P < 0.001$ ) than those obtained in the papaya D1 (14.32 ± 4.53%), control 1 (4188 bruchidae) and control 2 (0%) treatments. The average reduction rates obtained in the latter treatments were statistically identical to each other (Fig. 1).

The most effective doses in reducing *C. maculatus* adult numbers until day 120 of storage with neem were neem D2 and neem D3. Among the doses of papaya powder tested, dose D3 was more effective. Neem D2 and neem D3 were equally effective.

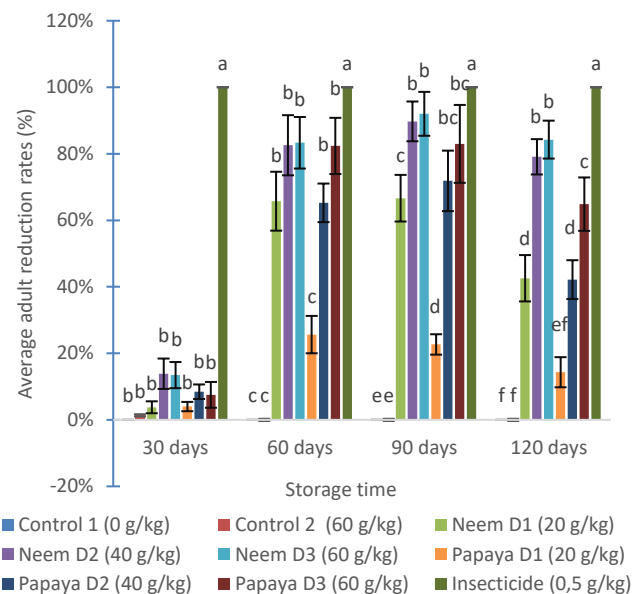


Fig. 1. Mean population reduction rates of *C. maculatus* adults in treated cowpea seeds after 30, 60, 90 and 120 days of storage. (For the same storage time, means with the same lower case letter are not significantly different according to the ANOVA followed by Student-Newman-Keuls test at the 5% threshold).

Both doses of neem were more effective in reducing the number of adult *C. maculatus* than papaya D3.

### B. Effect of treatments on the reduction of Cowpea seed mass loss

Cowpea seed mass loss increased significantly in the untreated control (Control 1) and in the control treated with cowpea seed powder (Control 2) compared to the other treatments and decreased with increasing biopesticide dose (Fig. 2).

In the treatment with the synthetic insecticide, no mass loss was recorded during the entire experiment. By day 30 of storage, the biopesticides used had no effect on reducing mass loss of cowpea seeds caused by *C. maculatus*. Mass losses in control 1 (3.75±1.7), control 2 (2.75±1.5), neem D1 (3±1.8), neem D2 (4±1.8), neem D3 (2.5±0.58), papaya D1 (4.5±3), papaya D2 (3.5±1.9), and papaya D3 (2.75±1.62) were statistically the same ( $P = 0.897$ ) (Fig. 2).

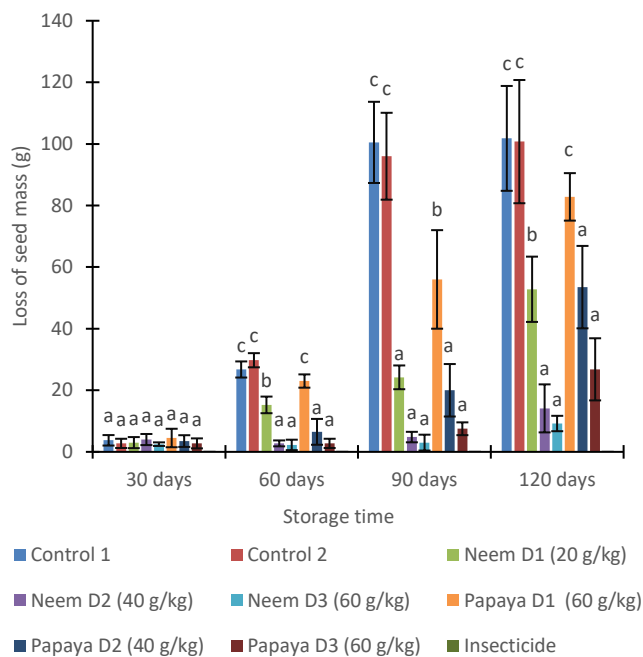


Fig. 2. Average loss ( $X \pm SD$ ) in cowpea seed mass as a function of storage time. (For the same shelf life, means with the same lower case letter are not significantly different according to the ANOVA followed by Student-Newman-Keuls test at the 5% threshold).

At day 60 of storage, the cowpea seed mass losses obtained in neem D2 ( $3.72 \pm 0.96$ ), neem D3 ( $2.25 \pm 1.7$ ), papaya D2 ( $6.5 \pm 4.2$ ) and papaya D3 ( $2.75 \pm 1.5$ ) treatments were significantly identical to each other ( $P = 0.249$ ), but statistically lower than the result in neem D1 ( $15.25 \pm 2.7$ ). In the neem D1 treatment, the mass loss obtained was significantly lower than the results obtained in the papaya D1 ( $23 \pm 2.16$ ), control 1 ( $26.75 \pm 2.62$ ) and control 2 ( $29.75 \pm 2.3$ ) treatments. The results obtained in the latter three treatments were statistically identical to one another (Fig. 2).

At day 90, cowpea seed mass losses in neem D1 ( $24.2 \pm 3.86$ ), neem D2 ( $4.8 \pm 1.7$ ), neem D3 ( $3 \pm 2.58$ ), papaya D2 ( $20 \pm 8.52$ ) and papaya D3 ( $7.5 \pm 2.08$ ) were statistically equivalent to one another ( $P = 0.467$ ), but significantly lower than in papaya D1 ( $56 \pm 16$ ). The result recorded in the papaya D1 treatment was statistically lower than the results obtained in control 1 ( $100.5 \pm 13.18$ ) and control 2 ( $96 \pm 14.11$ ). The results obtained in the latter two treatments were statistically identical to one another (Fig. 2).

On the 120th day of storage, the mass losses of cowpea seeds in the neem D2 ( $14.12 \pm 7.8$ ), neem D3 ( $9.2 \pm 2.5$ ), and papaya D3 ( $26.8 \pm 10.9$ ) treatments were statistically equivalent to one another, but significantly lower ( $P < 0.05$ ) than the losses obtained in the papaya D2 ( $53.5 \pm 13.38$ ) and neem D1 ( $52.8 \pm 10.6$ ) treatments. In the papaya D2 and neem D1 treatments, the losses recorded were significantly identical to each other, but lower than the mass losses obtained in the papaya D1, control 1 ( $101.8 \pm 17.03$ ) and control 2 ( $100.75 \pm 0.01$ ) treatments which were statistically equivalent (Fig. 2).

The most effective doses for reducing cowpea seed mass loss caused by *C. maculatus* with neem were D2 and D3. With papaya, D3 was the most effective. Neem D2, neem D3 and papaya D3 were equally effective in reducing cowpea seed mass loss.

### C. Assessment of treatment efficacy

The Weevil Perforation Index was estimated for each treatment after 120 days of storage. With the biopesticide products used, the perforation index decreases as dose increases (Table I). The lowest indices (18.14 and 13.79%) with neem were observed at 40 and 60 g/kg, respectively. With papaya, the lowest index (26.98%) was observed at 60 g/kg. All doses of the products used have a perforation index below 50% (Table I). All these treatments can be considered effective. The most effective being neem at 40 and 60 g/kg cowpea.

TABLE I: WEEVIL PERFORATION INDEX (WPI) ON DAY 120 OF STORAGE ACCORDING TO TREATMENTS

Doses (g/kg)	Control 2	Neem leaf Powder	Papaya leaf Powder	Insecticide
0	-	-	-	-
0.25	-	-	-	0%
20	-	37.43%	49.11%	-
40	-	18.14%	33.49%	-
60	50.18%	13.79%	26.98%	-

## IV. DISCUSSION

*C. maculatus* is a pest of cowpea stocks in Togo and West Africa. The use of neem and pawpaw leaf powders in different doses and for different lengths of time has shown its effectiveness in reducing populations of this weevil and the damage it causes (mass loss) to cowpea seeds. The effectiveness of these biopesticides is based on the results obtained from the first to the fourth months of storage. The results on the reduction of *C. maculatus* populations in the negative control and in the treatments with neem and papaya leaf powders on day 30 of storage were statistically identical. This result seems to be explained by the fact that the powders had no effect on the internal forms (larvae) of *C. maculatus* and the eggs. This corroborates the results obtained by N'Gombé [20] and Bawa [21] who used *Zanthoxylum zanthoxyloides* Lam. root bark powder (Rutaceae). These results are different from those obtained by Younoussa [18] who recorded at doses of 20 g/kg with neem leaf powder, a population reduction rate in *C. maculatus* in the first generation of  $88.23 \pm 3.29\%$ . This difference seems to be explained by the experimental method used. In our case, the treatment was carried out one week after infestation, whereas in his work, the treatment and the infestation were carried out at the same time.

After the 30th day of conservation, the effect of the products used became apparent. The results on population reduction showed a significant reduction in the population of *C. maculatus* with the products used compared to the negative control (control 1) and as a function of the doses. Indeed, the powders act by contact [22] and the bioactivity of these powders on the reduction seems to be due to the presence in the neem and papaya leaves of chemical compounds having insecticidal, oviposition inhibiting, fecundity, and fertility effects on the insect. The active principle that determines the inhibitory properties of neem leaves is azadirachtin, a triterpenoid that is toxic to many insects [23] and other compounds such as nimbine, salanine, meliandriol [24] that all act as anti-petals, repellents and have an insecticidal effect [16]. As for papaya leaves, the effect would

be due to papain, caricain and compounds such as chymopapain, glycyloendopeptidase [25]. The rate of bruchid reduction that increases with the dose of biopesticides seems to be explained by an increase in the amount of active ingredients. The reduction of *C. maculatus* population density by neem and papaya leaf powders was recorded after 60, 90 and 120 days of storage. The most effective treatment was neem leaf powder. Our results obtained on the population reduction rate of *C. maculatus* corroborate those of Alonmadon et al. [26] who used cowpea seed powder (*Pachyrhizus erosus*) at the same doses. However, our numbers of *C. maculatus* were higher than those obtained by Younoussa [18] who used neem leaf powder at lower doses of 2.5; 5; 10 and 20 g/kg cowpea. This difference may be due to the climatic conditions in the laboratory, the experimental methods which differ, the place and time of harvesting the leaves (which were harvested in our case in March 2020 in the field around the University of Lomé (Togo)) and also the period of the experiment. Moreover, the amount of azadirachtin in neem leaves can vary considerably with climatic conditions, soil composition, genotype, age and time of leaf harvesting [27], [28]. The reduction in population size of *C. maculatus* appears to be due to the anti-spotting, repellent and insecticidal effect of azadirachtin.

The Weevil Perforation Index (WPI) [19] obtained with the biopesticide treatments showed that these treatments are effective compared to the negative control (control 1).

Compared to the control (control 1), the biopesticides used contributed to the reduction of damage (seed perforation) and mass losses of cowpea stored for four months. Seed perforation and mass loss were observed much more in the negative control (control 1) and control 2 than in the treatments with neem and papaya leaf powders. *C. maculatus* weakly attacked cowpea seeds treated with neem leaf powder at high doses. Our results on the percentage of perforated seeds corroborate those obtained by Bawa [21] with *Z. zanthoxyloides* powder at 16 and 32 g/2 kg of cowpea.

The synthetic insecticide controlled the population size of *C. maculatus* better and reduced the damage (perforation and mass loss of cowpea seeds) caused by this weevil better than the biopesticides. These results obtained with the synthetic insecticide seem to be due to the dose (0.25 g/kg cowpea) used. Our results obtained with this insecticide corroborate those obtained by Younoussa [18] who used as synthetic insecticide Malagrain at the recommended dose of 0.5 g/kg but different from those obtained by Alonmadon et al. [26] who used pirimiphos-methyl + cyfluthrin at the dose of 0.5 g/kg.

## V. CONCLUSION

It appears from this study that all the biopesticides used were effective against *C. maculatus* adults especially at high doses (40, 60 g/kg cowpea for neem and 60 g/kg cowpea for papaya). With neem doses of 40 and 60 g/kg the population of *C. maculatus* is inhibited until day 90 of storage. With papaya leaf powder, however, this result was only achieved on the 60th day at a dose of 60 g/kg. The losses caused by *C. maculatus* on cowpea seeds are very low compared to control 1 with the high doses of the biopesticides used. Neem leaf powder was more effective than papaya leaf powder. The

effectiveness of neem leaf powder was 3 months at 40, 60 g/kg and that of papaya was 2 months at 60 g/kg. There was no significant difference in effect of neem at 40 g/kg and 60 g/kg.

In the interest of environmental and human health protection, neem leaf powder could therefore be used as an alternative to synthetic chemicals in the management of cowpea stock pests. With the availability of neem throughout Togo, neem leaves could be applied to protect cowpeas from *C. maculatus* attacks.

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