https://doi.org/10.33472/AFJBS.4.2.2022.77-85

ISSN: 2663-2187

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com


Research Paper

Open Access

# Insecticidal effect of powder and ethanolic extract of *Cnidoscolus aconitifolius* (Euphorbiaceae) on adults of *Callosobruchus maculatus* F. (Coleoptera: Bruchidae)

Lydia Hangnilo[1*], Haffizou Ganda[2] and Élisabeth T. Zannou[3]

[1]Laboratory of Agricultural Entomology (LEAg), Faculty of Agronomic Sciences, University of Abomey-Calavi, 03 BP 2819 Cotonou, Republic of Benin. E-mail: baloulydia@yahoo.fr

[2]Laboratory of Agricultural Entomology (LEAg), Faculty of Agronomic Sciences, University of Abomey-Calavi, 03 BP 2819 Cotonou, Republic of Benin. E-mail: gandahaffiz@gmail.com

[3]Laboratory of Agricultural Entomology (LEAg), Faculty of Agronomic Sciences, University of Abomey-Calavi, 03 BP 2819 Cotonou, Republic of Benin. E-mail: ezannou2@yahoo.fr

**Article Info**

Volume 4, Issue 2, April 2022

Received : 30 September 2021

Accepted : 15 February 2022

Published : 05 April 2022

doi: 10.33472/AFJBS.4.2.2022.77-85

## Abstract

Plants with insecticide effect is the main recourse to control cowpea weevil (*Callosobruchus maculatus*). This study assessed in laboratory, insecticidal effect of various doses of powder (125, 250, 375, 500, 625, 750 and 875 g of powder/kg of cowpea) and ethanolic extract (10%, 15%, 20% and 25% w/v) of *Cnidoscolus aconitifolius* on adults of the weevil. 10 adult couples of newly emerged weevil were introduced into each jar containing powder-seed or extract-seed mixture at a rate of 16 g healthy cowpea per treatment and per replication. The results revealed that the mortality rate was positively correlated with dose of powder (22.43 to 69.46%) and concentration of ethanolic extract (11.65 to 44.95%) of *C. aconitifolius*. Emergence rate decreased with dose of powder (12.31 to 5.13%) and concentration of ethanolic extract (19.24 to 8.44%). Infestation and oviposition rate were significantly reduced (Prob. < 0.001) by both extracts of *C. aconitifolius*. The different results revealed the potential of this plant to reduce the damage of *C. maculatus* in cowpea in storage. It is therefore possible to consider the use of extracts of this plant for the control of *C. maculatus*.

***Keywords:*** *Cowpea, C. maculatus, C. aconitifolius, Toxicity, Storage*

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## 1. Introduction

Growing food legumes constitute the best and least expensive solutions for feeding people in developing countries (Stoilova and Pereira, 2013). These ones produce nearly 90% of human consumption of legumes (Gordon, 2002). Among these legumes, cowpea (*Vigna unguiculata* F.) is one of the most important and the main source of protein for rural populations in Africa (Makanur *et al*., 2013; and CBDD, 2000). However, it is a host for pests of crops and stocks. Bruchids are insects that depredate cowpea stocks. They attack cowpea seed stocks, leading to quick crop damage. The losses caused by these ones in large cowpea storage systems

* *Corresponding author: Laboratory of Agricultural Entomology (LEAg), Faculty of Agronomic Sciences, University of Abomey-Calavi, 03 BP 2819 Cotonou, Republic of Benin. E-mail: baloulydia@yahoo.fr*

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are considerable. They are estimated at about 2.4% annual loss per ton of pods in storage (Kpatinvoh *et al*., 2016; Houinsou *et al*., 2014; and Zannou *et al*., 2000). Synthetic insecticides are the most use as control methods against these pests (Haubruge *et al*., 1988; and Relinger *et al*., 1988). Unluckly, this method has many drawbacks. In this perspective, plant-based pesticides are nowadays the recourse in integrated pest management (Isman, 1994; Regnault-Roger, 2002; and Ketoh *et al*., 2004). Many researchers have been done on the use of essential oils for the control of legumes pests (Johnson *et al*., 2006; and Kongue *et al*., 2018). Other plants that are still little studied are increasingly attracting the attention of researchers. This is the case of *Cnidoscolus aconitifolius* whose acaricidal effect of extracts on *Tetranychus urticae* Koch was proved by Numa *et al*. (2015). Faced with the need to protect cowpea stocks from bruchids, insecticidal properties of this plant could be explored. This justified this survey whose objective was to assess in laboratory, insecticidal effect of its extracts.

## 2. Materials and methods

The animal material consisted of adults of *Callosobruchus maculatus* (F.). Adults of this species were collected on cowpea seeds from a local market. These insects were maintained under laboratory conditions (T = 28.5 ± 2°C; RH = 70-77%) in mass rearing on cowpea seeds in glass jars of 1litre capacity. The plant material consisted of cowpea seeds (*Vigna unguiculata* F.) and dry leaves of *C. aconitifolius*. These ones were collected in Abomey-Calavi in subequatorial climate zone and certified under number YH 504 / HNB at the National Herbarium of Benin. An electric grinder (Binatone®, Model BLG 402, China) was used to grind the dried leaves into powder. Weighing was done on sensitive balance of precision 0.01 and brand AMPUT.

The ethanolic extract was obtained by maceration of the products of leaf grinding after drying under shade. Thus, 200 g of powder were weighed with a Sartorius® analytical balance and introduced into an Erlen-meyer flask to which two liters of ethanol were added. The mixture was mechanically shaken (cold) and brought to maceration. The macerate was filtered at the end of each 24 h for 72 h. The deposit is each time put back in maceration until the end of the 72 h. The filtrate obtained is evaporated with the rotavapor at 40°C.

The recovered extracts were placed in the oven at 45°C for drying. After complete drying, the extract obtained was stored in sterile, hermetically sealed glass vials.

### *2.1. Toxicity test*

The powder obtained from dry leaves of *C. aconitifolius* plant was mixed with 16 g of cowpea contained in glass jars, at doses of 2 g, 4 g, 6 g, 8 g, 10 g, 12 g and 14 g respectively. The contents of each jar were mixed so that all the seeds were covered by the powder. Under the same conditions, seeds received different concentrations of ethanolic extract (10%, 15%, 20% and 25%). One (1) ml of each concentration of ethanolic extract was added to 16 g of cowpea. These were thoroughly mixed by shaking to ensure uniform coating. The treated seeds were air-dried in shade for 30 min. Then 10 adult couples of newly emerged *C. maculatus* were introduced into each jar containing different doses of powder and different concentrations of ethanolic extract. Regular monitoring was done until death of the introduced couples of insects. Biological parameters such as mortality rate, infestation, oviposition and emergence rate were assessed. Mortality percentages of *C. maculatus* adults were calculated and corrected according to Abbott formula (Abbott, 1925).

## 3. Statistical analysis

A survival analysis was performed to study survival of insects over seven days according to dose of powder or ethanolic extract. Semi-parametric Cox model was then used to estimate effect of different formulations on insect mortality. Survival package (Terry, 2020) of R was used to implement Cox model. Beta regression was performed on infestation and emergence rates. Number of eggs was subjected to a simple linear regression. Threshold of significance is 5%. An analysis of variance (ANOVA) with two fixed factors is done on each of measured parameters to assess the insecticidal property.

## 4. Results

### *4.1. Effect of C. aconitifolius powder on mortality of C. maculatus*

The Table 1 shows a significant influence of treatment on insects' mortality. Indeed, mortality rates recorded with treatments T2, T3, T4, T5, T6 and T7 were significantly lower than that of control lot (Prob. < 0.001).

**Table 1 : Result of cox model applied to mortality rate of *C. maculatus* under the effect of powder of *C. aconitifolius***

| Modality | Coef | HR | Se(Coef) | HRinf | HRsup | Prob.(>\|z\|) |
|---|---|---|---|---|---|---|
| *C.aconitifolius* (Reference = T0; Wald Test = 261.9 df = 7; Prob = < 0.001) | | | | | | |
| Treatments T1 | 0.22 | 1.25 | 0.11 | 1.01 | 1.56 | 0.04 |
| Treatments T2 | 0.54 | 1.71 | 0.11 | 1.37 | 2.13 | < 0.001 |
| Treatments T3 | 0.55 | 1.73 | 0.11 | 1.39 | 2.15 | < 0.001 |
| Treatments T4 | 1.10 | 3.00 | 0.11 | 2.40 | 3.75 | < 0.001 |
| Treatments T5 | 1.18 | 3.25 | 0.12 | 2.59 | 4.08 | < 0.001 |
| Treatments T6 | 1.41 | 4.09 | 0.12 | 3.26 | 5.14 | < 0.001 |
| Treatments T7 | 1.20 | 3.32 | 0.11 | 2.66 | 4.14 | < 0.001 |

**Note:** Coef = Dose coefficient; HR = Hazard Ratio; Se: Standard error; HRinf and HRsup = lower and upper limits of the 95% confidence interval of the Hazard Ratio; Prob = probability.

Figure 1 indicates insect mortality rate is positively correlated with dose of *C. aconitifolius* powder and exposure time. The median survival time of *C. maculatus* under treatments T5, T6 and T7 was four days, while it is five days for insects under treatments T4, T3 and T2. Half of the insects in control lot (TO) were still alive on day six and half of those under T1 treatment were still alive on day five.

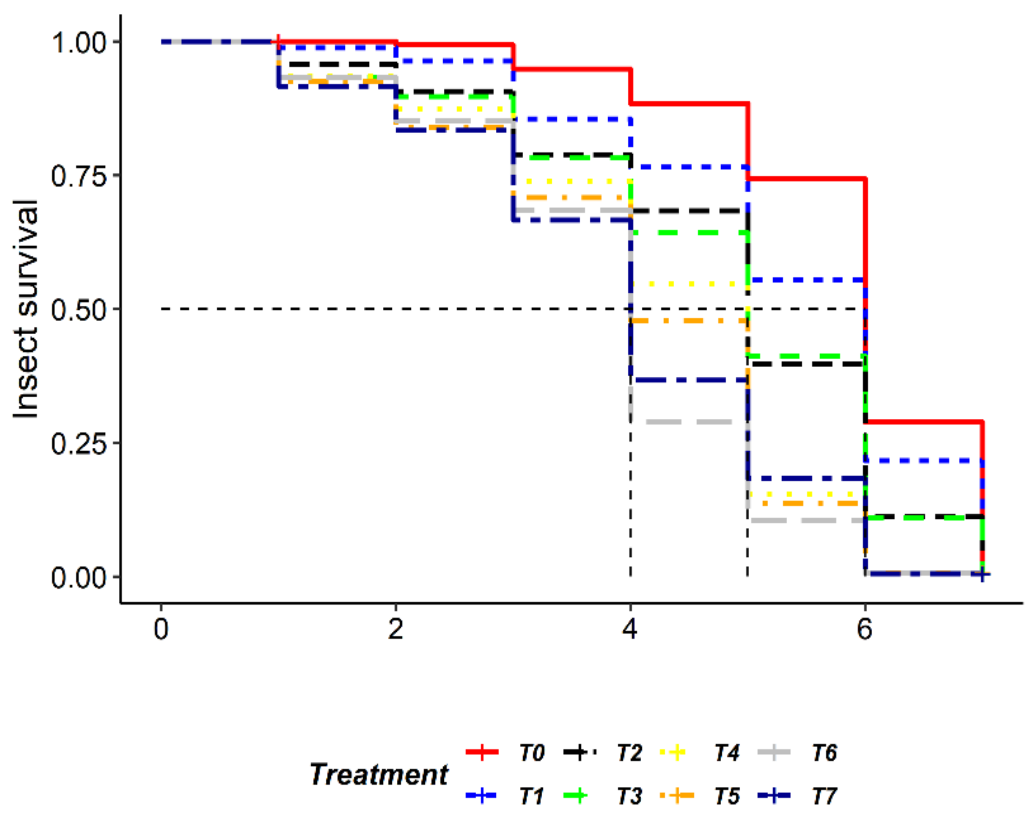


**Figure 1 : Survival curves of insects according to different treatments of *C. aconitifolius* powder**

### 4.2. Effect of ethanolic extract on mortality rate of *C. maculatus*

The Table 2 shows that treatment significantly reduced insect mortality (Prob = < 0.001).

**Table 2 : Result of cox model applied to mortality rate of *C. maculatus* under the effect of ethanolic extract of *C. aconitifolius***

| Modality | Coef | HR | Se(Coef) | HRinf | HRsup | Prob(>\|z\|) |
|---|---|---|---|---|---|---|
| *C.aconitifolius*(Reference = T0; Wald Test = 43.53 df = 5; Prob = < 0.001) | | | | | | |
| Treatments T1 | 0.14 | 1.15 | 0.16 | 0.84 | 1.58 | 0.38 |
| Treatments T2 | 0.17 | 1.18 | 0.16 | 0.86 | 1.62 | 0.29 |
| Treatments T3 | 0.59 | 1.81 | 0.16 | 1.32 | 2.48 | 0.001 |
| Treatments T4 | 0.84 | 2.32 | 0.16 | 1.69 | 3.18 | < 0.001 |
| Treatments TR | 0.07 | 1.08 | 0.16 | 0.79 | 1.48 | < 0.001 |

**Note:** Coef = Dose coefficient; HR = Hazard Ratio; Se: Standard error; HRinf and HRsup = lower and upper limits of the 95% confidence interval of the Hazard Ratio; Prob = probability.

Figure 2 shows median survival time under treatments T3 and T4 was four days and five days for insects subjected to treatments T0, TR, T1 and T2.

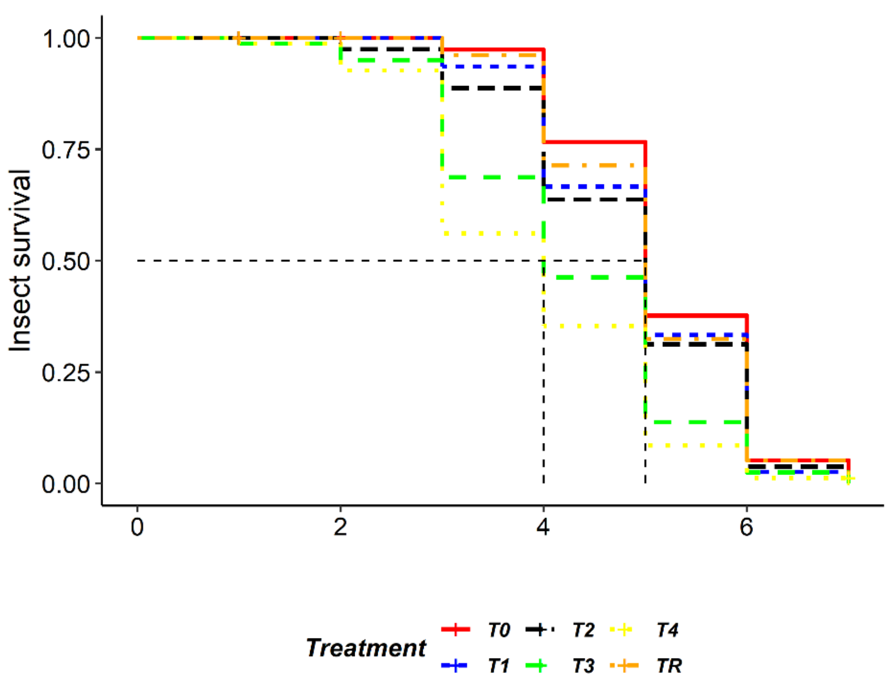


**Figure 2 : Survival curves of insects according to different treatments with ethanolic extract of *C. aconitifolius***

### 4.3. Effect of treatments on the infestation rate of *C. maculatus*

The analyses of variance indicated that treatments had a significant effect ($p < 0.05$) on infestation rate of cowpea seeds. However, there was no significant difference between the infestation rates obtained with the control treatment and doses 2 g, 4 g and 6 g of powder (Table 3).

The Table 4 indicates that treatment with ethanolic extract significantly reduced the infestation rate (Prob < 0.05).

**Table 3 : Result of beta regression applied to infestation rate of *C. maculatus* under the effect of powder of *C. aconitifolius***

| Powder (Infestation rate) | *C. aconitifolius* | Pseudo R² = 0.876 |
|---|---|---|
| **Treatment** | **Average (Standard deviation)** | **Prob (>\|z\|)** |
| T0 (Reference) | 0.98(0.01)d | <0.001 |
| T1 | 0.98(0.01)d | 0.99 |
| T2 | 0.97(0.01)d | 0.28 |
| T3 | 0.95(0.01)cd | 0.01 |
| T4 | 0.91(0.01)bc | <0.001 |
| T5 | 0.84(0.02)ab | <0.002 |
| T6 | 0.83(0.02)a | <0.003 |
| T7 | 0.83(0.02)a | <0.004 |

**Note:** Values with the same letters in indices are not significantly different from each other for the same type of powder. Prob = probability of significance.

**Table 4 : Result of beta regression applied to infestation rate of *C. maculatus* under the effect of ethanol extract of *C. aconitifolius***

| Ethanolic extract (Infestation rate) | *C. aconitifolius* | Pseudo R² = 0,99 |
|---|---|---|
| **Treatment** | **Average (Standard deviation)** | **Prob (>\|z\|)** |
| TR (Reference) | 0.99(0.001)e | <0.001 |
| T0 | 0.99(0.001)e | 0.99 |
| T1 | 0.58(0.005)d | <0.001 |
| T2 | 0.55(0.005)c | <0.001 |
| T3 | 0.48(0.005)b | <0.001 |
| T4 | 0.41(0.005)a | <0.001 |

**Note:** Values with the same letters in indices are not significantly different from each other for the same type of powder. Prob = probability of significance.

### 4.4. Effect of treatments on fecundity of C. maculatus

The Table 5 shows that average number of eggs decreased with increasing powder dose. Powder dose statistically influenced the number of eggs (Prob < 0.05). It is the same analysis for treatments with the ethanolic extract (Table 6).

**Table 5 : Result of linear regression applied to fecundity of *C. maculatus* under the effect of powder of *C. aconitifolius***

| Powdee (Fertility) | *C. aconitifolius* | Pseudo R² = 0.42 |
|---|---|---|
| **Treatment** | **Average (Standard deviation)** | **Prob (>\|z\|)** |
| T0 (Reference) | 56.76(2.491)c | <0.001 |
| T1 | 54.85(2.491)c | 0.58936 |

| Table 5 (cont.) | | |
|---|---|---|
| **Powdee (Fertility)** | ***C. aconitifolius*** | **Pseudo R² = 0.42** |
| **Treatment** | **Average (Standard deviation)** | **Prob (>\|*z*\|)** |
| T2 | 52.46(2.491)bc | 0.22735 |
| T3 | 50.3(2.491)abc | 0.07190 |
| T4 | 47.2(2.491)abc | 0.00881 |
| T5 | 41.79(2.491)ab | <0.001 |
| T6 | 39.43(2.491)a | <0.001 |
| T7 | 39.94(2.491)a | <0.001 |

**Note:** Values with the same letters in indices are not significantly different from each other for the same type of powder. Prob = probability of significance.

**Table 6 : Result of linear regression applied to fecundity of *C. maculatus* under the effect of ethanolic extract of *C. aconitifolius***

| **Ethanolic extract (Fertility)** | ***C. aconitifolius*** | **Pseudo $R^2$ = 0.94** |
|---|---|---|
| **Treatment** | **Average (Standard deviation)** | **Prob (>\|*z*\|)** |
| TR (Reference) | 57.25(0.96)c | <0.001 |
| T0 | 56.88(0.96)c | 0.78 |
| T1 | 21.75(0.96)b | <0.001 |
| T2 | 15.65(0.96)a | <0.001 |
| T3 | 14.23(0.96)a | <0.001 |
| T4 | 13.6(0.96)a | <0.001 |

**Note:** Values with the same letters in subscripts are not significantly different from each other for the same type of extract. Prob = probability of significance.

### *4.5. Effect of treatments on emergence of adults of C. maculatus*

The Table 7 indicates that powder dose statistically influenced emergence rate (Prob < 0.05). It is the same analysis for treatments with ethanolic extract (Prob < 0.05) Table 8.

**Table 7 : Result of beta regression applied to emergence of *C. maculatus* under the effect of powder of *C. aconitifolius***

| **Powder (Emergence rate)** | ***C. aconitifolius*** | **Pseudo $R^2$ = 0.95** |
|---|---|---|
| **Treatment** | **Average (Standard deviation)** | **Prob (>\|z\|)** |
| T0 (Reference) | 0.99(0.002)d | <0.001 |
| T1 | 0.99(0.002)d | 0.99 |
| T2 | 0.99(0.002)d | 0.54032 |
| T3 | 0.98(0.003)d | 0.00784 |

Table 7 (Cont.)

| **Powder (Emergence rate)** | ***C. aconitifolius*** | **Pseudo $R^2$ = 0.95** |
|---|---|---|
| **Treatment** | **Average (Standard deviation)** | **Prob (>\|z\|)** |
| T4 | 0.93(0.005)c | <0.001 |
| T5 | 0.9(0.006)b | <0.001 |
| T6 | 0.9(0.006)b | <0.001 |
| T7 | 0.74(0.009)a | <0.001 |

**Note:** Values with the same letters in indices are not significantly different from each other for the same type of powder. Prob = probability of significance.

**Table 8 : Result of beta regression applied to emergence of *C. maculatus* under the effect of ethanolic extract of *C. aconitifolius***

| **Ethanolic extract (Emergence rate)** | ***C. aconitifolius*** | **Pseudo $R^2$ = 0.94** |
|---|---|---|
| **Treatment** | **Average (Standard deviation)** | **Prob (>\|z\|)** |
| TR (Référence) | 0.26(0.01)d | <0.001 |
| T0 | 0.27(0.01)d | 0.61 |
| T1 | 0.19(0.009)c | <0.001 |
| T2 | 0.14(0.008)b | <0.001 |
| T3 | 0.1(0.007)a | <0.001 |
| T4 | 0.08(0.006)a | <0.001 |

**Note:** Values with the same letters in indices are not significantly different from each other for the same type of powder. Prob = probability of significance

## 5. Discussion

Prior to this work, no studies tested the insecticidal effect of *C. aconitifolius* extracts on *C. maculatus* adults. Indeed, studies have tested the acaricidal effect of *C. aconitifolius* on *Tetranychus urticae* (Numa *et al*., 2015). According to these authors, ethanolic extract of *C. aconitifolius* has remarkable acaricidal properties on *Tetranychus urticae* females.

The present study tested the insecticidal effect of the powder and ethanolic extract of *C. aconitifolius* on adults of *C. maculatus* under laboratory conditions. This study showed that powder and ethanolic extract of *C. aconitifolius* reduced seed infestation, life span, fecundity and emergence rate of *C. maculatus*. This reduction was much greater at higher doses. The most effective treatment was the ethanolic extract, for which values of the studied parameters were very low. The results recorded were comparable to those of Dabiré (1993), Nuto (1995), Kétoh (1998) and Johnson (2006) whose researches showed that certain plant species reduce the life span of adults and the fecundity of female *C. maculatus*. They also agree with Gakuru and Foua-Bi (1995) who report that plant species are very effective in reducing the life span of *C. maculatus* and *Sitophilus orizae*. Thus, the decrease in insect fecundity would be due to early death caused by respiratory intoxication related to the volatile compounds contained in powder and ethanolic extract of the plant (Schmidt *et al*., 1991; Koumaglo *et al*., 1996; Glitho *et al*., 1997; Mazibur and Gerhard, 1999; and Séri-Kouassi, 2004).

## 6. Conclusion

The powder and ethanolic extract of *C. aconitifolius* have significantly reduced the life span of *C. maculatus*. Different treatments reduced seed infestation, fecundity, and insect emergence rate. The different trials revealed that ethanolic extract is more effective than the powder. Extracts of *C. aconitifolius* can be used in the control of *C. maculatus*, in cowpea stocks in Benin.

**Ethical opinion**

The execution of this research work has received the favorable opinion of scientific committee of Doctoral School (FAST / UAC) under the number (UAC / FAST / EDSVT / 574601).

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**Cite this article as:** Lydia Hangnilo, Haffizou Ganda and Élisabeth T. Zannou (2022). Insecticidal effect of powder and ethanolic extract of *Cnidoscolus aconitifolius* (Euphorbiaceae) on adults of *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). *African Journal of Biological Sciences.* 4(2), 77-85. doi: 10.33472/AFJBS.4.2.2022.77-85.