**International Journal of Biology Research**
**ISSN: 2455-6548; Impact Factor: RJIF 5.22**
**Received: 01-08-2019; Accepted: 03-09-2019**
**www.biologyjournal.in**
**Volume 4; Issue 4; October 2019; Page No. 01-03**

# Survival potentials of *Callosobruchus maculatus* (F) on cowpea seeds treated with leaf and root bark of *Calotropis procera* and *Parquetina nigrescens.*

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**Abstract**
This study investigated the survival potential of *Callosobruchus maculatus* reared on cowpea seeds treated with different concentrations of powder and solvent extracts of the leaf and root bark of *Calotropis procera* and *Parquetina nigrescens*. These were with a view to developing a new bio-control of *Callosobruchus maculatus.* Different concentrations of the powder and solvent extract of the plant materials were prepared and used for survivorship bioassay. The results showed that there was relatively low (5 – 25%) survival of second instar larvae of *C. maculatus* treated with 5% concentration of ethanolic and methanolic extracts of leaf and root bark of *C. procera* and *P. nigrescens.* The adults of *C. maculatus* treated at 5% concentration of ethanolic and methanolic extracts of leaf and root bark of *C. procera* and *P. nigrescens* had no survival except for the ethanolic and methanolic extract of *C. procera* root bark where 20 and 35% survival were recorded respectively. The study concluded that the leaf and root bark of *C. procera* and *P. nigrescens* contained bioactive substances that could reduce survival rate of *C. maculatus*.

**Keywords:** *calotropis procera, parquetina nigrescens, callosobruchus maculatus*

## 1. Introduction
*Callosobruchus maculatus*, an insect pest of cowpea commonly known as the cowpea weevil, or bean beetle (Tran and Credland, 1995) [1]. *C. maculatus* is a member family of Chrysomelidae. The insect has been reported to be a world threat of cowpea seed (Fricke and Arnqvist, 2007) [2]. However, various measures including largely the use of gaseous fumigants and residual contact insecticides that are hazardous have been employed to protect cowpea seed from infestation so as to ensure safe storage of the products (El-Aziz, 2011) [3]. In several countries, the control of stored grains against insect infestation which is primarily achieved by mixing of synthetic insecticides such as phosphine (common name: phosphine gas, trade name: eco2fume) and bromo methane (common name: methyl bromide, trade names: embafume and terabol) with stored products is no longer permitted (Asawalam and Adesiyan, 2001) [4]. This is due to human health hazards and environmental concerns (Tapondjou *et al*., 2002) [5], problem of insect resistance to synthetic insecticides and mammalian toxicity due to residue persistence (Obembe and Kayode, 2013) [6]. Hence, there is an urgent need for safer alternatives in lieu of conventional insecticides. The use of locally available plant materials to reduce insect damage to stored grains is being carried out in traditional farm storage in most developing countries (Hassanali *et al*., 1990) [7].
The potency of plant materials and their effects on survival of *C. maculatus* reared on cowpea treated with powder and solvent extracts leaf and root bark of *Calotropis procera* and *Parquetina nigrescens* were studied.

## Materials and Methods
The leaf and root bark of *C. procera* and *P. nigrescens* were collected from Iyara village in Kogi State, Nigeria. The leaf and root bark were rinsed with water, dried in the oven at 40$^{o}$C for 48 hours, ground to a powder with an electrical blender (USHA MG 2053 N). Thirty grams (30 g) each of the powder of leaves and root bark of *C. procera* and *P. nigrescens* were soaked in 300 ml of absolute ethanol and methanol solvents separately in flasks for 6 hours, stirring intermittently with a sterile glass rod, then, filtered through What man No.4 filter paper. The solvents were removed in a water bath at 79$^{0}$ C for ethanol and 66$^{0}$C for methanol and the residue was re-dissolved in the least amount of ethanol and methanol to obtain a stock solution of 40 mg mL$^{-1}$ concentration (Ali and Elgimabia, 2015) [8]. Different desirable concentrations of the plant extracts (1%, 2%, 3%, 4%, and 5%) were prepared for use in this study (Warthen *et al*., 1984; Ranjini and Nambiar, 2015) [9, 10]
Different dosages (1.25%, 2.5%, 5%, 7.5%, 10% w/w) of the dried powder of leaf and root bark of *C. procera* and *P. nigrescens* were mixed separately with 40g each of cowpea seeds in Petri-dishes and twenty (1-3 day- old) adults and second instar larvae insects each were introduced to each treatment in a separate experiment The Petri-dishes were perforated and covered with muslin cloths to allow air circulation. The same procedure was repeated for solvent extracts, 5 mL of 1%, 2% 3%, 4%, and 5% of methanolic and ethanolic extracts of leaf and root bark of *C. procera* and *P. nigrescens* were used to treat 40 g each of cowpea seeds and air dried for 1 hour according to Eziah *et al.* (2013) [11]. To 40 g each of treated cowpea seeds, twenty (1-3 day- old) adults and second instar larvae insects each were introduced to each treatment separately. Percentage survivorship was recorded after 72 h.

$$\% \text{ survivorship} = \frac{\text{Total number of insect introduced - number of dead insects}}{\text{Total number of insect introduced}} \text{x } 100$$

**Data analysis.**
Data obtained was subjected to analysis of variance (ANOVA) procedure of Minitab 16.1 (2007) [12]. Tukey's Test at P = 0.05 was used to compare means.

## Results

The result of the percentage survival of the second instar larvae of *C. maculatus* on treated cowpea with different concentrations of powder of leaf and root bark of *C. procera* and *P. nigrescens* after 72 h of exposure is shown in Table 1. The larval survivorship obtained for the various plant powders was between 15-100%. Larval survivorship across the powder of leaf and root bark of *C. procera* and *P. nigrescens* shows significant (P<0.05) diffrernce.

Percentage survival of the adults of *C. maculatus* on treated cowpea with the powder of leaf and root bark of *C. procera* and *P. nigrescens* after 72 h of exposure are shown in Table 2. The highest (55%) number of survival percentage of the adult of *C. maculatus* at 10% powder was recorded with the powder extract of *P. nigrescens* root bark and lowest (35%) with powder of *C. procera* leaf. Across the concentrations, larval survivorship increases with decrease in concentration. There was significant ($p < 0.05$) difference between the powder of leaf and root bark of *C. procera* and *P. nigrescens*. The adult survivorship recorded in all the four different powder ranged from 35-100%.

The percentage survival of the second instar larvae of *C. maculatus* on treated cowpea with different concentrations of ethanolic and methanolic extracts of leaf and root bark of *C. procera* and *P. nigrescens* after 72 h of exposure are presented in the Table 3. A decrease in concentration resulted in increase in percentage survival in all the plant extracts investigated. Survival of larvae at 5% concentration was greatly affected as survival was recorded only with the ethanolic extract of *C. procera* root bark (20%) and methanolic extract of *C. procera* root bark (35%). However, survival at the concentration of 1%, 2%, 3% and 4% ranged between 85-95%, 55-8 5%, 25-70% and 5-50%, respectively with significant difference between them (P<0.05).

The percentage survival of the adults of *C. maculatus* on treated cowpea with different concentrations of ethanolic and methanolic extracts of leaf and root bark of *C. procera* and *P. nigrescens* after seventy-two hours of exposure is shown in the Table 4. The survival of the adults of *C. maculatus* increases with decrease in concentration. Survival at concentration of 5% varied from 5 – 25% while at 3% and 4%, a relatively higher survival was recorded. The highest survival was recorded at 1% concentration. The percentage survivorship among the extracts at different concentrations was significantly different (P< 0.05).

**Table 1:** Percentage survivorship of the second instar larvae of *C. maculatus* treated with powder of leaf and root bark of *C. procera* (Cp ) and *P. nigrescens* (Pn) after 72h exposure

| Plant materials | No. of insect used | Percentage survivorship (%) per gram weight of dry powder | | | | |
|---|---|---|---|---|---|---|
| | | 1.2 | 2.5 | 5 | 7.5 | 10 |
| *C. procera* leaf powder | 20 | 95.00±0.00$^{b}$ | 85.00±0.00$^{b}$ | 60.00±5.00$^{b}$ | 35.00±10.00$^{d}$ | 15.00±10.00$^{c}$ |
| *C. procera* root bark powder | 20 | 100.00±0.00$^{a}$ | 90.00±5.00$^{a}$ | 70.00±5.00$^{a}$ | 50.00±5.00$^{b}$ | 35.00±10.00$^{a}$ |
| *P. nigrescens* leaf powder | 20 | 95.00±5.00$^{b}$ | 85.00±5.00$^{b}$ | 55.00±5.00$^{c}$ | 45.00±10.00$^{c}$ | 25.00±10.00$^{b}$ |
| *P. nigrescens* root bark powder | 20 | 95.00±0.00$^{b}$ | 85.00±0.00$^{b}$ | 70.00±5.00$^{a}$ | 55.00±5.00$^{a}$ | 35.00±10.00$^{a}$ |

Means in the same column with the same alphabets are not significantly different (P<0.05).

**Table 2:** Percentage survivorship of the adults of *C. maculatus* on cowpea treated with powder of leaf and root bark of *C. procera* (Cp) and *P. nigrescens* (Pn) after 72 h exposure

| Plant materials | No. of insect used | Percentage survivorship (%) per gram weight of dry powder | | | | |
|---|---|---|---|---|---|---|
| | | 1.25 | 2.5 | 5 | 7.5 | 10 |
| *C. procera* leaf powder | 20 | 95.00±0.00$^{b}$ | 90.00±5.00$^{b}$ | 75.00±0.00$^{b}$ | 55.00±5.00$^{c}$ | 35.00±5.00$^{d}$ |
| *C. procera* root bark powder | 20 | 100.00±0.00$^{a}$ | 90.00±5.00$^{b}$ | 75.00±0.00$^{b}$ | 60.00±5.00$^{b}$ | 45.00±5.00$^{c}$ |
| *P. nigrescens* leaf powder | 20 | 100.00±0.00$^{a}$ | 90.00±0.00$^{b}$ | 75.00±5.00$^{b}$ | 65.00± 0.00$^{a}$ | 50.00±5.00$^{b}$ |
| *P. nigrescens* root bark powder | 20 | 100.00±0.00$^{a}$ | 95.00±0.00$^{a}$ | 85.00±0.00$^{a}$ | 65.00±0.00$^{a}$ | 55.00±0.00$^{a}$ |

Means in the same column with the same alphabets are not significantly different (P<0.05).

**Table 3:** Percentage survivorship of the second instar larvae of *C. maculatus* on cowpea treated with ethanolic and methanolic extracts of leaf and root bark of *C. procera (Cp)* and *P. nigrescens (Pn)* after 72 h exposure

| Plant materials | No. of insect used | Percentage mortality (%) per plant extract concentration (g ml$^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| *C. procera* leaf ethanolic extract | 20 | 85.00±0.00$^{c}$ | 75.00±10.00$^{b}$ | 40.00±5.00$^{d}$ | 10.00±5.00$^{c}$ | 0.00±0.00$^{c}$ |
| *C. procera* leaf methanolic extract | 20 | 90.00±0.00$^{b}$ | 75.00±10.00$^{b}$ | 45.00±0.00$^{c}$ | 15.00±10.00$^{b}$ | 0.00±0.00$^{c}$ |
| *C. procera* root bark ethanolic extract | 20 | 90.00±0.00$^{b}$ | 85.00±0.00$^{a}$ | 65.00±10.00$^{b}$ | 50.00±10.00$^{a}$ | 20.00±5.00$^{b}$ |
| *C. procera* root bark methanolic extract | 20 | 95.00±5.00$^{a}$ | 85.00±0.00$^{a}$ | 70.00±5.00$^{a}$ | 50.00±5.00$^{a}$ | 35.00±10.00$^{a}$ |
| *P. nigrescens* leaf ethanolic extract | 20 | 95.00±5.00$^{a}$ | 65.00±10.00$^{d}$ | 25.00±10.00$^{e}$ | 5.00±0.00$^{d}$ | 0.00±0.00$^{c}$ |
| *P. nigrescens* leaf methanolic extract | 20 | 85.00±5.00$^{c}$ | 55.00±5.00$^{e}$ | 40.00±5.00$^{d}$ | 5.00±5.00$^{d}$ | 0.00±0.00$^{c}$ |
| *P. nigrescens* root bark ethanolic extract | 20 | 85.00±0.00$^{c}$ | 70.00±5.00$^{c}$ | 40.00±5.00$^{d}$ | 5.00±0.00$^{d}$ | 0.00±0.00$^{c}$ |
| *P. nigrescens* root bark methanolic extract | 20 | 90.00±0.00$^{b}$ | 75.00±5.00$^{b}$ | 45.00±0.00$^{c}$ | 10.00±5.00$^{c}$ | 0.00±0.00$^{c}$ |

Means in the same column with the same alphabets are not significantly different (P<0.05).

**Table 4:** Percentage survivorship of the adults of *C. maculatus*on cowpea treated with ethanolic and methanolic extracts of leaf and root bark of *C. procera* (Cp) and *P. nigrescens* (Pn) after 72h exposure

| Plant materials | No. of insect used | Percentage survivorship (%) per plant extract concentration (g ml$^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| *C. procera* leaf ethanolic extract | 20 | 80.00±5.00$^{c}$ | 80.00±5.00$^{b}$ | 55.00±5.00$^{d}$ | 30.00±5.00$^{d}$ | 5.00±0.00$^{c}$ |
| *C. procera* leaf methanolic extract | 20 | 95.00±0.00$^{a}$ | 85.00±0.00$^{a}$ | 60.00±5.00$^{c}$ | 40.00±5.00$^{c}$ | 15.00±10.00$^{b}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| *C. procera* root bark ethanolic extract | 20 | 95.00±0.00[a] | 85.00±0.00[a] | 65.00±0.00[b] | 45.00±10.00[b] | 25.00±10.00[a] |
| *C. procera* root bark methanolic extract | 20 | 95.00±5.00[a] | 85.00±10.00[a] | 70.00±5.00[a] | 50.00±5.00[a] | 25.00±10.00[a] |
| *P. nigrescens* leaf ethanolic extract | 20 | 90.00±5.00[b] | 75.00±0.00[c] | 45.00±10.00[f] | 15.00±10.00[g] | 5.00±0.00[c] |
| *P. nigrescens* leaf methanolic extract | 20 | 90.00±0.00[b] | 75.00±10.00[c] | 50.00±5.00[e] | 20.00±5.00[f] | 5.00±0.00[c] |
| *P. nigrescens* root bark ethanolic extract | 20 | 95.00±0.00[a] | 80.00±5.00[b] | 55.00±5.00[d] | 25.00±0.00[e] | 5.00±0.00[c] |
| *P. nigrescens* root bark methanolic extract | 20 | 95.00±0.00[a] | 80.00±0.00[b] | 63.33±10.40[c] | 30.00±5.00[d] | 5.00±0.00[c] |

Means in the same column with the same alphabets are not significantly different (P<0.05).

## Discussion

The low survival of adult and larvae in the treated seeds confirms the activity of toxic secondary metabolites present in the extracts, which may suppress development and survival of the adult and larvae of *C. maculatus* and these insecticidal secondary metabolites may include catechin, gallocatechin, diterpenes and sesquiterpenes (Neves and Camara, 2012) [13]. These phytochemical constituents vary from plant to plant and from one plant part to another (Neeharika *et al.*, 2012; Kawo *et al.*, 2013) [14, 15]. The relative amount of phytochemical substances from plant extract depends on the solubility of the phytochemical in the solvent used for the extraction (Olowosulu and Ibrahim, 2006; Akinyemi and Dada, 2013) [16, 17]. This amount of phytochemical constituents present in ethanolic extracts may be more than the methanolic extract which probably accounted for the higher efficacy of ethanolic extracts of leaf and root bark of *C. procera* and *P. nigrescens* over methanolic extracts as observed in the study.

However, the survival of adults and larvae of *C. maculatus* as observed in this study may be due to a modified behavior that may make the insects to avoid contact with treated surfaces (Silva *et al.*, 2013) [18]. In addition, insects perceive the presence of insecticide in the environment through the learning ability of insect and genetic modifications in their peripheral receptors or central processing systems, leading to the evolution of behavioral resistance to insecticides in some species (Gould, 1991; Silva *et al.*, 2013) [19, 18].

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