

Toxicity effect of powder, ethanolic and methanolic extracts of leaf and root bark of *C. procera* and *P. nigrescens* on the adult and second instar larvae of *C. maculatus* (Coleoptera: Chrysomelidae)

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Abstract

This study assessed the toxicity effects of powder and solvent extracts of the leaf and root bark of *Calotropis procera* and *Parquetina nigrescens* on the mortality of *Callosobruchus maculatus* treated with powder and extracts of the leaf and root bark of the plants. These were with a view to developing a new biological method of control of the insect pest. Different concentrations of the powder and solvent extract leaf and root bark of *C. procera* and *P. nigrescens* were used for toxicity test on adults and second instar larvae of *C. maculatus*. The results showed that second instar larvae of *C. maculatus* treated with the ethanolic and methanolic extracts of leaf and root bark of *C. procera* and *P. nigrescens* at 5% concentration had 100% mortality for various plant extracts except for the extracts of *C. procera* root bark where mortality ranged between 70 to 85%. The mortality of the adults of *C. maculatus* was 100% in all the ethanolic and methanolic extracts of leaf and root bark of *C. procera* and *P. nigrescens* at 5% concentration.

Keywords: *Callosobruchus maculatus*, instar larvae.

1. Introduction

The use of synthetic organic insecticides poses hazards to the environment and development of resistant strains, hence, the need to explore suitable alternative products for the control of insect pest. Screening of plant materials that are considered to be eco-friendly for their insecticidal activities offer a promising approach to solving the problem of infestation of food crops by insect pest during postharvest storage ((Arnason *et al.*, 1989; Rahman and Schmidt, 1999) [1, 2].

Insecticidal properties of many plant parts such as leaf, root bark and seeds against several insects have been demonstrated (Overgaard *et al.*, 2014) [3]. One of the approaches in the search of novel biological insecticides entails screening of plant materials for their deleterious effects on insects (Isman, 1995) [4]. Leaf as well as seed extracts of several plants have been reported to have toxic and potent growth reducing activity to insects (Champagne *et al.*, 1993) [5]. The admixture of certain aromatic leaves, fruits or plant extracts (Apuuli and Villet, 1996; Appel *et al.*, 1999) [6,7] can reduce the need for, and risks associated with, the use of insecticides.

Calotropis procera and *Parquetina nigrescens* (Gentianale: Apocynaceae) are milky latex producing plant (Raginee *et al.*, 2010) [8]. The latex of *C. procera* contains caoutchouc, calotropin, uscharin 0.45 %, calotoxin 0.15 %, calactin (composed of calotropagenin and hexose) 0.15 %, trypsin, voruscharin, uzarigenin, syriogenin and proceroside (Hanna *et al.*, 2002) [9]. Root bark possesses phenolics benzoyllineolone, benzoyl isolineolone, madaralban and madar fluavil (Khanzada *et al.*, 2008) [10]. Bioactivity of the plant such as molluscidal (Hussein *et al.*, 1994) [11] and nematocidal (Rakesh *et al.*, 2001) [12] had been reported.

There are no studies so far on the control of agricultural pests by *P. nigrescens* except its toxicity bioassays on

household pest; *Periplaneta americana* (Ogunleye, 2010) [13]. However, *Thevetia nerifolia* belonging to the same family of Apocynaceae with *P. nigrescens* had been used to control insects such as *Helicoverpa armigera* (Mishra *et al.*, 2015) [14]; *C. maculatus* (Mollah and Islam, 2007) [15]; and *Spodoptera litura* (Upadhyay, 2013) [16].

The present research work was undertaken to investigate the toxicity effect of powder, ethanolic and methanolic extracts of leaf and root bark of *C. procera* and *P. nigrescens* on the mortality of adult and second instar larvae of *C. maculatus*

Materials and Methods

The toxicity of the powder extracts of the leaf and root bark of *C. procera* and *P. nigrescens* was determined according to Chougourou *et al.* (2015) [17]. The different dosages (1.25 %, 2.5 %, 5 %, 7.5 %, 10 % w/w) of powder obtained from dried leaves and root bark of *C. procera* and *P. nigrescens* were mixed separately and gently with twenty (1-3 day- old) adults and second instar larvae in Petri dishes and 40g each of cowpea seeds were added to each treatments. For each set of treatment, non-treated insects were used as control. The Petri-dishes were perforated and covered with muslin cloths to allow air circulation. To twenty (1-3 day- old) adults and second instar larvae insects, one microliter of different concentrations (1 %, 2 %, 3 %, 4 %, and 5 %) of methanolic and ethanolic extracts of leaf and root bark of *C. procera* and *P. nigrescens* was applied to the dorsal surface of the thorax of each insect using a micropipette (Eziah *et al.*, 2013) [18]. These were transferred into clean, perforated and netted Petri dishes lined with filter paper containing 40g of cowpea seeds. Non treated insects were used as control. Each treatment was replicated three times. Mortality was recorded after 72 hours of treatment, insects were considered dead if they fail to respond to prodding with a blunt probe and the percentage mortality was determined as

below:

$$\% \text{ mortality} = \frac{\text{Total number of insect introduced} - \text{number of live insects}}{\text{Total number of insect introduced}} \times 100$$

Data analysis

Data obtained was subjected to analysis of variance (ANOVA) procedure of Minitab 16.1 (2007) [19]. Tukey’s Test at P = 0.05 was used to compare means.

Results

The percentage mortality of the second instar larvae of *C. maculatus* treated with the powder of leaf and root bark of *C. procera* and *P. nigrescens* after 72 h of exposure is presented in the Table 1. Larval mortality across the powder of leaf and root bark of *C. procera* and *P. nigrescens* showed significant difference (P< 0.05). Powder of *C. procera* leaf recorded the highest larval mortality at different concentrations, followed by the powder of *P. nigrescens* leaf, while the lowest mortality percentage was recorded with the powder extract of *C. procera* root bark.

Table 2 shows the result of the mortality of the adults of *C. maculatus* treated with different dosages of powder of leaf and root bark of *C. procera* and *P. nigrescens* after seventy-two hours of exposure. At the different dosages of powder of leaf and root bark of *C. procera* and *P. nigrescens*, powder of *C. procera* leaf recorded the highest percentage mortality, followed by powder extract of *C. procera* root bark and the least with the powder of *P. nigrescens* root bark. There was significant difference (P < 0.05) in all the plant powder at each concentration.

The percentage mortality of the second instar larvae of *C. maculatus* on treated cowpea with different concentrations of the ethanolic and methanolic extracts of leaf and root bark of *C. procera* and *P. nigrescens* after 72 h of exposure is shown in Table 3. Percentage mortality of the second instar larvae of *C. maculatus* decreases with decrease in

concentration across the treatments. At 5 % concentration, a mortality percentage of 100 % was recorded for all the extracts, except for the ethanolic and methanolic extracts of *C. procera* root bark which recorded 85 % and 70 % respectively. The percentage mortality of second instar larvae of *C. maculatus* at concentration of 4% ranged between 50 to 100 %. However, ethanolic and methanolic extract of *C. procera* root bark recorded 60 % and 50 % respectively. Mortality percentage at 3 % concentration was averagely 65 % across the plant extracts, with the highest (80 %) in ethanolic extract of *P. nigrescens* leaf and 35 % with the methanolic extract of *C. procera* root bark. There was a considerable decrease in percentage mortality at 2 % and 1% ranging between 20-40 % and 10-20 % respectively. The percentage mortalities across the plant extracts were significantly different (P< 0.05) at each concentration.

Table 4 depicts percentage mortality of the adult 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 exposure. Mortality decreased as the concentration of plant extract decreases. Ethanolic extract of *C. procera* leaf, *P. nigrescens* leaf and root bark recorded 100 % mortality at concentration of 5 %, while at the same 5 % concentration of methanolic extract of *C. procera* leaf, ethanolic extract of *C. procera* root bark and methanolic extract of *C. procera* root bark, mortality was 90 %, 80 % and 75 % respectively. At concentration of 4%, mortality ranged from 55 % to 90 %. The highest percentage mortality of adult was recorded in the ethanolic extract of *P. nigrescens* leaf, followed by the methanolic extract of *P. nigrescens* leaf, with the least mortality recorded in the methnolic extract of *C. procera* root bark. Percentage of adult mortality at concentration of 1 %, 2 % and 3 %, ranged between 10-25 %, 20-30 %, and 35-60 % respectively. Morttality of the extracts at different concentrations was significantly (p<0.05) different except at 2 % concentration.

Table 1: Mortality 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 mortality (%) per gram weight of dry powder				
		1.25	2.5	5	7.5	10
<i>C. procera</i> leaf powder	20	10.00±0.00 ^a	20.00±0.00 ^a	45.00±5.00 ^a	70.00±10.00 ^a	90.00±10.00 ^a
<i>C. procera</i> root bark powder	20	5.00±0.00 ^b	15.00±5.00 ^b	35.00±5.00 ^a	55.00±5.00 ^c	70.00±10.00 ^c
<i>P. nigrescens</i> leaf powder	20	10.00±0.00 ^a	20.00±5.00 ^a	40.00±5.00 ^b	60.00±10.00 ^b	80.00±10.00 ^b
<i>P. nigrescens</i> root bark powder	20	10.00±0.00 ^a	20.00±0.00 ^a	35.00±5.00 ^a	50.00±5.00 ^d	70.00±10.00 ^c

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

Table 2: Mortality of the adults of *C. maculatus* 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 mortality (%) per gram weight of dry powder				
		1.25	2.5	5	7.5	10
<i>C. procera</i> leaf powder	20	10.00±0.00 ^a	15.00±5.00 ^a	30.00±0.00 ^a	50.00±10.00 ^a	65.00±5.00 ^a
<i>C. procera</i> root bark powder	20	5.00±0.00 ^b	15.00±5.00 ^a	30.00±0.00 ^a	45.00±5.00 ^b	60.00±10.00 ^b
<i>P. nigrescens</i> leaf powder	20	5.00±0.00 ^b	15.00±0.00 ^a	25.00±5.00 ^b	40.00±0.00 ^c	55.00±5.00 ^c
<i>P. nigrescens</i> root bark powder	20	5.00±0.00 ^b	10.00±0.00 ^b	20.00±0.00 ^a	40.00±10.00 ^a	50.00±10.00 ^d

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

Table 3: Percentage mortality of the second instar larvae of *C. maculatus* 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 mortality (%) per plant extract concentration (g ml ⁻¹)				
		1	2	3	4	5
<i>C. procera</i> leaf ethanolic extract	20	20.00±0.00 ^a	30.00±10.00 ^c	65.00±5.00 ^b	95.00±5.00 ^b	100.00±0.00 ^a
<i>C. procera</i> leaf methanolic extract	20	15.00±0.00 ^b	30.00±10.00 ^c	60.00±0.00 ^c	90.00±10.00 ^c	100.00±0.00 ^a
<i>C. procera</i> root bark ethanolic extract	20	15.00±0.00 ^b	20.00±0.00 ^d	40.00±10.00 ^d	60.00±10.00 ^d	85.00±5.00 ^b
<i>C. procera</i> root bark methanolic extract	20	10.00±5.00 ^c	20.00±0.00 ^d	35.00±5.00 ^e	55.00±5.00 ^e	70.00±10.00 ^c
<i>P. nigrescens</i> leaf ethanolic extract	20	10.00±5.00 ^c	40.00±10.00 ^a	80.00±10.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
<i>P. nigrescens</i> leaf methanolic extract	20	20.00±5.00 ^a	30.00±5.00 ^c	65.00±5.00 ^b	95.00±5.00 ^b	100.00±0.00 ^a
<i>P. nigrescens</i> root bark ethanolic extract	20	15.00±0.00 ^b	35.00±5.00 ^b	65.00±0.00 ^b	100.00±0.00 ^a	100.00±0.00 ^a
<i>P. nigrescens</i> root bark methanolic extract	20	15.00±0.00 ^b	30.00±5.00 ^c	60.00±0.00 ^c	90.00±5.00 ^c	100.00±0.00 ^a

Table 4: Percentage mortality of the adults of *C. maculatus* 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
<i>C. procera</i> leaf ethanolic extract	20	10.00±0.00 ^c	25.00±5.00 ^b	50.00±5.00 ^c	75.00±5.00 ^d	100.00±0.00 ^a
<i>C. procera</i> leaf methanolic extract	20	10.00±0.00 ^c	20.00±0.00 ^c	45.00±5.00 ^d	65.00±5.00 ^e	90.00±10.00 ^b
<i>C. procera</i> root bark ethanolic extract	20	10.00±0.00 ^c	20.00±0.00 ^c	40.00±0.00 ^c	60.00±0.00 ^f	80.00±10.00 ^c
<i>C. procera</i> root bark methanolic extract	20	10.00±0.00 ^c	20.00±5.00 ^d	35.00±5.00 ^f	55.00±5.00 ^g	75.00±5.00 ^d
<i>P. nigrescens</i> leaf ethanolic extract	20	15.00±5.00 ^b	30.00±0.00 ^a	60.00±10.00 ^b	90.00±10.00 ^a	100.00±0.00 ^a
<i>P. nigrescens</i> leaf methanolic extract	20	15.00±0.00 ^b	30.00±5.00 ^a	55.00±0.00 ^b	85.00±5.00 ^b	100.00±0.00 ^a
<i>P. nigrescens</i> root bark ethanolic extract	20	10.00±0.00 ^c	25.00±5.00 ^b	50.00±5.00 ^c	80.00±0.00 ^c	100.00±0.00 ^a
<i>P. nigrescens</i> root bark methanolic extract	20	25.00±0.00 ^a	25.00±0.00 ^b	50.00±5.00 ^c	75.00±5.00 ^d	100.00±0.00 ^a

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

Discussion

The milky sap of *C. procera* is a mixture of various chemicals such as calotropin, calotoxin, calactin, uscharidin, voruscharin which are caustic in nature and are considered poisonous (Alencar *et al.*, 2006) [20]. Accidental exposure to the latex of *C. procera* has been reported to cause inflammation of the skin and eyes (Al-Mezaine *et al.*, 2005) [21].

The toxicity effect of ingestion of fresh *C. procera* leaves and latex has been reported (Singhal and Kumar, 2009) [22]. Phytochemical extracts of *C. procera* leaves indicated the presence of alkaloids, saponins, phenols, tannins, and flavanoids which are known to contain pesticidal and medicinal properties (Verma *et al.*, 2013) [23]. According to Badshah *et al.* (2004) [24], the chemical poisons from *C. procera* are mostly alkaloids. Alkaloids are plant products, which are nitrogenous in nature are heterocyclic compounds having strong effects on the nervous system of animals and which may result in death.

Chemical compounds such as calotropagenin, flavonoids, and saponins have been reported to be present in the latex of *C. procera* (Kanojiya and Madhusudan, 2012) [25] and cardenolides in the *C. procera* latex are associated with the toxicity effects (Elgamal *et al.*, 1999) [26]. Saponins are known to disrupt cellular membranes (Osborn, 1996) [27]. The present study shows that the extracts of leaf and root bark of *C. procera* and *P. nigrescens* were effective against *C. maculatus*. Mortality increases with concentration of extracts of leaf and root bark of *C. procera* and *P. nigrescens*. This suggests that the normal process of growth and development can be inhibited by plant extracts. Further developmental stages were not conspicuously observed in second instar larvae treated with the extracts of leaf and root bark of *C. procera* and *P. nigrescens* (Anita *et al.*, 2012) [28]. Interference of bioactive compounds in the extracts with the

molting process may be responsible for the lack of development (Deka and Singh, 2005) [29]. The solvent extracts and powder of leaf and root bark *C. procera* and *P. nigrescens* demonstrated toxicity against the adults and larvae of *C. maculatus*. These findings agree with a similar study by Ogunwolu and Odunlami (1996) [30] on the toxicity of the powder of *Z. zanthoxyloides* root bark on the adults of *C. maculatus*. The toxicity activity of the extracts and powder of leaf and root bark *C. procera* and *P. nigrescens* in this study may be caused by the bioactivity of their chemical constituents and physical action of the formulations (Denloye *et al.*, 2010) [31].

The bioactive constituents of the plant materials contain active ingredients which exhibit potential to cause interference with the normal metabolism of insect (Sripongpun, 2008) [32] and seems to be more in the ethanolic extract which caused the higher mortality recorded in solvent extracts as compared to powder extracts (Iram *et al.*, 2013) [33]. The powder extract of plant materials has toxicity effects on *C. maculatus* (Adesina *et al.*, 2015) [34]. The powder used in this study led to insect mortality due to their physical action on respiration through the blockage of the spiracles of the insects (Ofuya and Dawodu, 2004) [35] and abrasion of insect cuticle which may lead to water loss, stress and death (Sousa *et al.*, 2005) [36]. Leaf extract of *C. procera* is quite effective as larvicides and a better alternative for insect control (Nighat *et al.*, 2010) [37]. Alkaloid constituent of the latex of *C. procera* plant was shown to contain insecticidal properties (Larhsini *et al.*, 1997) [38]. The results of the work of Hiren *et al.* (2011) [39] on ethanolic leaf extract of *C. procera* showed a significant larvicidal activity against 3rd instar larvae of *A. stephensi* which exhibited 100% mortality at 2000 ppm after 48 hours of incubation is consonance with the results of the present study. The toxicity of *C. procera* and *P. nigrescens* may be used safely in high doses (Ramos *et al.*, 2006) [40].

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