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Ovicidal potentials of *Calotropis procera* and *Parquetina nigrescens* on *Callosobruchus maculatus* (F) (Coleoptera: Chrysomelidae)

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Abstract
This study evaluated the ovicidal potentials of the powder and solvent extracts of the leaf and root bark of *Calotropis procera* and *Parquetina nigrescens* on the ovipositional activities of *Callosobruchus maculatus.* These were with a view to developing a novel biological method of mitigating the problem of postharvest losses of cowpea seeds in storage due to infestation by *C. maculatus*. Different concentrations of powder, ethanolic and methanolic extracts of leaf and root bark of *C. procera* and *P. nigrescens* were prepared and used for oviposition and hatchability tests of eggs of *C. maculatus*. The results showed that the percentage reduction in number of eggs laid decreases with decrease in concentration of the plant extracts. The ethanolic extract of *P. nigrescens* leaf had the highest (69%) percentage reduction in number of eggs laid. Hatching percentage was lowest at 5% plant extract concentration and highest at 1% extract concentration and was significant ($P< 0.05$). The study concluded that the leaf and root bark of *C. procera* and *P. nigrescens* posses antiovipositional chemical compounds that could be used to control cowpea seeds infestation by bean weevil.

Keywords: oviposition, concentration, leaf, root bark, *Callosobruchus maculatus*

Introduction
Bean weevil, *Callosobruchus maculatus* is among the major insect pests of legumes which causes high infestation of cowpea both on the field and in storage (Kang *et al.*, 2013) [1]. Infestation of cowpea usually commences in the field and progress to storage where proliferation of the pest occurs rapidly (Deshpande *et al.*, 2011; Soundararajan *et al*., 2012) [2, 3]. During postharvest storage, female bean weevil after copulation lays eggs on cowpea seeds in which larval to pupal stage occurs within the cowpea seeds where they cause enormous damage by feeding on the cowpea seeds (Ileke *et al.*, 2013) [4].
Control of insect pests of cowpea seeds in storage is largely on the use of synthetic chemical insecticides which have negative impingement on the environment, non-target organisms and human health. Hence, there is a need to develop cheap, safe and readily available materials from botanical extracts. Plants are considered rich sources of bioactive chemicals and may be an alternative source of insect control agents (Silva *et al.*, 2013) [5].
The objective of the present study was to investigate the ovicidal effects of powder, ethanolic and methanolic extracts of leaf and root bark of *Calotropis procera* and *Parquetina nigrescens* on *C. maculatus*.

Materials and Methods
Cowpea seeds of 40 g each were treated with powder (1.25 %, 2.5 %, 5 %, 7.5 %, 10 % w/w) of leaf and root bark of *C. procera* and *P. nigrescens.* The same procedure was repeated for solvent extracts, 5 mL of 1 %, 2 % 3 %, 4 %, and 5 % of methanol and ethanol 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) [6]. Five copulating pairs of adult *C. maculatus* 1-3 days old *C. maculatus* were introduced into each Petri-dish containing treated cowpea seeds. The Petri-dishes were perforated and covered with muslin cloths to allow air circulation. There was also a control treatment containing untreated cowpea seeds. The number of eggs laid by the female beetles on the cowpea seeds was recorded after seven days and the number of hatched eggs was recorded after fourteen days. This was used to calculate the percentage of hatched eggs and the percentage reduction of laid egg respectively, as follows:

$$\% \text{ reduction of laid egg} = \frac{\text{Number of laid eggs in the control} - \text{Number of laid eggs in the treatment}}{\text{Number of laid eggs in the control}} \times 100$$

$$\text{Egg hatching (\%)} = \frac{\text{Number of hatched eggs}}{\text{Number of total laid eggs}} \times 100$$

Data Analysis
Data obtained was subjected to analysis of variance (ANOVA) procedure of Minitab 16.1 (2007) [7]. Tukey's Test at P = 0.05 was used to compare means.

Results
The percentage reduction in the number of laid eggs of *C. maculatus on cowpea* seeds treated with powder of leaf and root bark of *C. procera* and *P. nigrescens* is presented in Table 1. There was significant difference ($P< 0.05$) at each concentration in all the powder. Powder of *C. procera* leaf recorded the highest reduction in oviposition (10 - 15.10 %) and the lowest in powder of *P. nigrescens* root bark (2 – 7.2 %).

Table 2 shows the percentage reduction in the number of laid eggs of *C. maculatus on cowpea* seeds treated with ethanolic and methanolic extracts of leaf and root bark of *C. procera* and *P. nigrescens*. The percentage reduction in number of laid eggs decreases with decrease in concentration of the plant extracts. At all the concentration of extracts tested, reduction in the number of laid eggs was more in the ethanolic extracts of leaf and root extracts of *C. procera* and *P. nigrescens* than their corresponding methanolic extracts. Ethanolic extract of *P. nigrescens* leaf recorded the highest reduction in number of laid eggs (69 %) at 5 % concentration and lowest (15 %) in methanolic extract of *P. nigrescens* root bark at 1 % concentration. There was significant difference ($P< 0.05$) at each concentration in all the extracts.

Table 3 shows the results of the effects of powder of leaf and root bark of *C. procera* and *P. nigrescens* on the hatchability of the eggs of *C. maculatus.* There was significantly difference ($P< 0.05$) at each concentration in all the powder. Powder of *P. nigrescens* root bark recorded the highest hatchability at each concentration tested, while the powder of *C. procera* leaf recorded the lowest.

Table 4 shows the effects of ethanolic and methanolic extracts of leaf and root bark of *C. procera* and *P. nigrescens* on the hatchability of the eggs of *C. maculatus*. At all percentage concentration of extracts, more eggs hatched in the methanolic extracts of leaf and root extracts of *C. procera* and *P. nigrescens* than their corresponding ethanolic extracts. Egg hatching was highest at all concentrations in ethanolic extract of *P. nigrscens* root bark compared to other extracts tested. There was significant difference ($P< 0.05$) at each concentration in all the extracts. Number of eggs hatched increased with decrease in concentration of the extracts.

Discussion

In this study, the solvent extracts and powder of leaf and root bark *C. procera* and *P. nigrescens* demonstrated ovicidal activity against the adults of *C. maculatus.* It appears that the extracts of leaf and root bark of *C. procera* and *P. nigrescens* possess oviposition deterrent properties. Oviposition deterrence may be due to the changes induced in physiology and behaviour in the adult of *C. maculatus* and this could be reflected in their egg laying capacity (Shifa vanmathi *et al.,* 2010) [8]. The findings of this study agree with a similar study by Ogunwolu and Odunlami (1996) [9], where root bark powder of *Z. zanthoxyloides* caused fewer eggs to be laid and a corresponding smaller number of eggs that hatched may result (Raja *et al.,* 2000; Rao and Sharma, 2007; Vanmathi *et al.,* 2012) [10, 11, 12]. This is because toxic substances present in leaf and root bark extracts may penetrate chorion and suppressed further embryonic development (Raja *et al.,* 2001) [13].

The report of Adesina, *et al.* (2015) [14] on the antioviposition effects of leaf powder extract of *Xylopia aethiopica* and *Aframomum meleguta* on *C. maculatus* is similar to the results of this present work. The extract of leaf and root bark *C. procera* and *P. nigrescens* likely contain specific inhibitors such as trypsin inhibitor that may cause reduction in the number of hatched eggs by binding to the chorion of the eggs during embryonic period and cause inhibition of proteases needed for the hydrolysis of egg chrion protein (Young *et al.,* 1997; Nath *et al.,* 2015) [15, 16]. The ovicidal and hatching inhibitory properties (Anita *et al.,* 2012) [17] of the extracts of leaf and root bark of *C. procera* and *P. nigrescens* may also penetrate the chorion of eggs and creates a hypercarbic condition within eggs, a condition of abnormally elevated carbon dioxide (CO_2) levels in the egg (DonPedro, 1989; Ajayi, 2013) [18, 19].

Table 1: Reduction effects of powder of leaf and root bark of *C. procera* (Cp) and *P. nigrescens* (Pn) on the number of laid eggs of *C. maculatus*.

Plant materials	Percentage reduction (%) in number of laid eggs per gram weight of dry powder				
	1.25	2.5	5	7.5	10
C. procera leaf powder	10.00±1.00^{a}	10.00±1.00^{a}	11.00±1.00^{a}	12.20±0.10^{a}	15.10±0.10^{a}
C. procera root bark powder	2.30±0.10^{c}	3.00±1.00^{c}	5.00±1.00^{c}	7.30±0.10^{c}	9.01±0.01^{c}
P. nigrescens leaf powder	5.00±1.00^{b}	5.00±1.00^{b}	7.20±0.10^{b}	10.10±0.10^{b}	12.00±1.00^{b}
P. nigrescens root bark powder	2.00±0.00^{c}	2.00±1.00^{c}	4.20±0.10^{c}	6.00±1.00^{d}	7.20±0.10^{d}

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

Table 2: Reduction effects of ethanolic and methanolic extracts of leaf and root bark of *C. procera* (Cp) and *P. nigrescens* (Pn) on the number of laid eggs of *C. maculatus*.

Plant materials	Percentage reduction (%) in number of laid eggs per plant extract concentration (g ml^{-1})				
	1	2	3	4	5
C. procera leaf ethanolic extract	48.10±0.10^{b}	51.40±0.10^{b}	55.10±0.10^{b}	58.30±0.10^{b}	60.10±0.10^{b}
C. procera leaf methanoicl extract	25.00±2.00^{f}	27.20±0.10^{f}	29.10±0.10^{f}	32.00±1.00^{f}	35.00±1.00^{f}
C. procera root bark ethanolic extract	34.50±0.20^{d}	36.30±0.10^{d}	39.20±0.20^{d}	41.60±0.10^{d}	42.00±1.00^{d}
C. procera root bark methanolic extract	18.10±0.10^{g}	20.10±0.00^{g}	21.00±1.00^{g}	23.40±0.20^{g}	24.60±0.20^{g}
P. nigrescens leaf ethanolic extract	62.00±2.00^{a}	63.70±0.20^{a}	66.50±0.30^{a}	67.00±2.00^{a}	69.00±1.00^{a}
P. nigrescens leaf methanolic extract	30.70±0.20^{c}	33.10±0.10^{e}	35.10±0.10^{e}	38.10±0.10^{e}	40.20±0.20^{e}
P. nigrescens root bark ethanolic extract	43.30±0.10^{c}	44.40±0.10^{c}	46.00±2.00^{c}	50.10±0.10^{c}	53.30±0.10^{c}
P. nigrescens root bark methanolic extract	15.10±0.10^{h}	18.40±0.20^{h}	20.10±0.10^{g}	22.00±2.00^{g}	22.00±1.00^{h}

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

Table 3: Effects of extracts of leaf and root bark of *C. procera* (Cp) and *P. nigrescens* (Pn) on the hatchability of the eggs of *C. maculatus*.

Plant materials	Percentage hatchability (%) per gram weight of dry powder				
	1.25	2.5	5	7.5	10
C. procera leaf powder	91.10±0.10^{d}	88.20±0.10^{d}	85.70±0.10^{d}	82.50±0.10^{d}	74.00±1.00^{d}
C. procera root bark powder	96.90±0.10^{b}	94.00±1.00^{b}	91.10±0.10^{b}	87.00±1.00^{b}	82.50±0.10^{b}
P. nigrescens leaf powder	95.10±0.10^{c}	91.20±0.10^{c}	88.00±1.00^{c}	84.10±0.10^{c}	78.00±1.00^{c}
P. nigrescens root bark powder	98.00±1.00^{a}	96.00±1.00^{a}	94.00±1.00^{a}	90.20±0.1^{a}	85.10±0.10^{a}

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

Table 4: Effects of ethanolic and methanolic extracts of leaf and root bark of *C. procera* (Cp) and *P. nigrescens* (Pn) on the hatchability of the eggs of *C. maculates*

Plant materials	Percentage hatchability (%) per plant extract concentration (g ml^{-1})				
	1	2	3	4	5
C. procera leaf ethanolic extract	14.00±1.00^{g}	13.20±0.10^{g}	12.80±0.10^{e}	11.50±0.20^{f}	10.00±1.00^{f}
C. procera leaf methanolic extract	32.00±1.00^{c}	30.10±0.10^{c}	29.30±0.10^{b}	26.40±0.30^{b}	24.00±1.00^{b}
C. procera root bark ethanolic extract	20.10±0.10^{e}	17.90±0.10^{e}	16.20±0.1^{d}	15.24±0.01^{d}	14.30±0.10^{d}
C. procera root bark methanolic extract	35.10±0.10^{b}	32.30±0.10^{b}	29.70±0.2^{b}	27.00±1.00^{b}	25.00±1.00^{b}
P. nigrescens leaf ethanoicl extract	8.00±1.00^{h}	7.89±0.10^{h}	7.00±1.00^{f}	6.10±0.10^{g}	5.20±0.20^{g}
P. nigrescens leaf methanolic extract	27.90±0.10^{d}	26.10±0.10^{d}	24.00±1.00^{c}	22.90±0.10^{c}	21.20±0.10^{c}
P. nigrescens root bark ethanolic extract	16.10±0.10^{f}	14.60±0.20^{f}	13.70±0.10^{e}	12.80±0.10^{e}	12.00±1.00^{e}
P. nigrescens root bark methanolic extract	39.20±0.10^{a}	37.10±0.10^{a}	35.73±0.42^{a}	33.00±1.00^{a}	31.60±0.10^{a}

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

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