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ORIGINAL RESEARCH ARTICLE

Laboratory evaluation of three underutilized Nigerian plants as cowpea seeds protectants against cowpea beetle, *Callosobruchus maculatus* (Fab.) [Coleoptera: Chrysomelidae]

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Abstract

Powders and methanolic extracts of *Clerodendrum capitatum*, *Phyllanthus fraternus* and *Tithonia diversifolia* were evaluated for their insecticidal activities against *Callosobruchus maculatus* (Fab.). Plant materials were tested for contact toxicity, oviposition and adult emergence at rates of 0.5, 1.0, 2.0, 4.0 and 5.0 g (w/w) for plant powders and 0.1, 0.2, 0.5, 1.0 and 2.0 ml/20 g (v/w) of cowpea seeds for plant methanolic extracts. The results of the plants powders showed that *T. diversifolia* was the most effective as it caused 55.0, 60.0, 67.5, 100.0% and 100.0% mortality at the concentrations of 0.5, 1.0, 2.0, 4.0 and 5.0 g/20 g of cowpea seeds within 24 h of application. The least effective plant powder was *C. capitatum* which evoked the insect mortality of 77.50% at rate 5.0 g within 24 h of exposure. Similar trend of results were obtained on the plant methanolic extracts. Extracts caused more mortality, prevented oviposition and emergence of adult cowpea beetle, more than the plant powders. The calculated lethal dose (LD_{50} and $_{90}$) and concentrations (LC_{50} and $_{90}$) of the plant powders and methanolic extracts showed that *T. diversifolia* had the lowest values while *C. capitatum* had the highest across all period of exposure. *Tithonia diversifolia* methanolic extract completely inhibited oviposition and adult emergence at concentrations 0.2–2.0 ml. *Tithonia diversifolia* powder and methanolic extract were found to be the most effective in protecting cowpea seeds against *C. maculatus*. This can be a better alternative to synthetic insecticides since it is abundant in our environment.

Keywords Entomocide · Mortality · Oviposition · *Callosobruchus maculatus* · *Clerodendrum capitatum* · *Phyllanthus fraternus* · *Tithonia diversifolia*

Introduction

Cowpea (*Vigna unguiculata* L.) is a major food legume cultivated in tropical and subtropical countries where it forms the essential component of agriculture (Fatokun et al. 2002). Cowpea is the most widely used multipurpose and nutritious grain legumes to combat malnutrition in young children (Singh 1985). Cowpea is a rich source of dietary proteins, vitamins and minerals (Akinkurolere 2012). It has been one of the main staple components of the human diet, especially in the developing countries where animal protein is in limited supply (Singh 1978; 1985; Singh and Jackai 1985).

Insect pests pose a major threat to cowpea production and storage of grains in tropical countries, directly affecting the food security programmes. The cowpea beetle, *C. maculatus* (Fab.) is a cosmopolitan field-to-store pest of cowpea (Akinkurolere 2012). The larvae feed through the pod cover and remain concealed within the developing seeds (Southgate 1978). When such seeds are harvested and stored, the

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insect continues to feed as hidden infestation and emerges as an adult and may cause destruction within 3–4 months, thereby rendering the seed unfit for human consumption as well as reduction in seed viability leading to poor germination (Singh and Jackai 1985). It causes quantitative as well as qualitative losses. This insect causes severe losses up to 100% in unprotected cowpea (Singh 1978; 1985; Ogunwolu and Odunlami 1996; Akinkurolere et al. 2006; Akinkurolere 2012).

Farmers over the years have relied on the use of synthetic chemical insecticides and fumigants to control stored product insects (Obembe and Kayode 2013; Idoko and Ileke 2020; Ileke et al 2020a; b; 2021a). The use of insecticides on stored grains is associated with several problems leading to health hazards and very expensive for the poor resource farmers to apply. This practice is also associated with the poor knowledge of application and the non-availability of the chemicals when needed. All these problems have led to searching for safe, ecologically tolerant and cheap control measures (Akinkurolere et al. 2006; Oni and Ileke 2008; Adedire et al. 2011; Ileke and Oni 2011; Oni 2011; Ileke et al. 2021b). Recent findings have revealed that plant oils, plant extracts and dry powders of different plant parts are effective protectants for stored cowpeas (Ogunwolu and Odunlami 1996; Rajapakse and van Emden 1997; Lale and Abdulrahman 1999; Boeke et al. 2001; Akinkurolere et al. 2006; Akinkurolere 2007; Ileke et al. 2012; 2013).

Clerodendrum capitatum (Willd.) is an indigenous tropical Africa perennial shrub with rapid growth, erect, and well branched, grows up to 0.5–2 m high (Houngnon et al. 2008). In some parts of Africa the leaves of the plant are traditionally used in the treatment of malaria. Based on ethnobotanical report in the maritime region of Togo, *C. capitatum* is frequently used for the treatment of high blood pressure (hypertension) and also to alleviate obesity, jaundice and constipation. In Nigeria, the plant is used to treat diabetes mellitus, obesity and high blood pressure (Adeneye et al. 2008). Despite the widespread use of *C. capitatum*, there is lack of experimental data on its possible toxicity. *Phyllantus fraternus* (Webster) is a monoecious, annual herb of up to 45–60 cm tall and in the tropical region of the world from the family Euphobiaceae (Mehta et al. 2013). A common weed found abundantly during the rainy season (Khan and Khan 2004) and spreads widely in West Africa where they are utilized in traditional medicine to cure ailments (Umoh et al. 2013). It hepatoprotective properties had been reported (Rastogi and Mahrotra 1990). In Nigeria, the Yorubas referred to it as "eyin olobe", Hausa as "geeron tsutsaayee" and Igbo as "Ite knwonwa nazu" and in English as "leaf flower" or "chamber bitter" (Etukudo 2003; Okujagu et al. 2005; Adesina et al. 2014). Nigerians used it infusion for health maintenance (Etukudo 2000) but the insecticidal properties against coleopterans are scarce in literature.

Tithonia diversifolia (Hemsl.) is a species of flowering plant in the Asteraceae family that is commonly referred to as the tree marigold or Mexican sunflower (Jama et al. 2000; Adedire and Akinneye 2004). Tree marigold is 2–3 m (6.6–9.8 ft) in height with upright and sometimes ligneous stalks in the form of woody shrubs (Jama et al. 2000). This plant is a weed that grows rapidly and has become an option reasonably priced alternative to expensive synthetic fertilizers (Jama et al. 2000). Tree marigold is an introduced weed with a fast expanding range in Nigeria (Adedire and Akinneye 2004). It is commonly found along major highways in Southwestern Nigeria and it has been observed to exhibit allelopathic effect on Siam weed, *Chromolaena odorata* (Adedire and Akinneye 2004). This present study sought to protect stored cowpea seeds from *C. maculatus* infestation using powders and methanolic extracts of *C. capitatum, P. fraternus* and *T. diversifolia*.

Materials and methods

Collection of cowpea seeds

Clean, uninfested, cowpea (*Vigna unguiculata*) seeds, variety Ife brown (3 kg) was used for this research work and it was purchased from the Ministry of Agriculture (Agricultural Development Programme unit), Akure, Ondo State, Nigeria. The cowpea seeds were first sterilized by putting them in a deep freezer set at −5 °C for 72 h to get rid of all the existing eggs and larvae. This process was carried out to eliminate all the life stages of *C. maculatus* particularly the eggs which are susceptible to low temperature (Koehler 2003). The disinfected cowpea seeds were later spread on a clean sheet to dissipate absorbed moisture and to prevent mould growth (Adedire and Ajayi 1996).

Insect culture

The parent stock of *C. maculatus* used for this research work was collected from naturally infested cowpea seeds from Storage Entomology Research Laboratory, Department of Biology, Federal University of Technology Akure (7°18′5.84 N, 5°8′3.19E), Ondo State, Nigeria. About 500 g of clean disinfected seeds was weighed using an electronic weighing balance (Model JTC 2101 N) into 1 L glass kilner jar. Thereafter, twenty (10 males: 10 females) newly emerged adult beetles were introduced into the kilner jar. The kilner jar was covered with muslin cloth held with rubber band to allow easy flow of air and to stop the insects from escaping (Ileke et al. 2012). The insect culture was kept in the laboratory for 35 days to allow the insects to oviposit and multiply (Adedire et al. 2011). The new adults that emerged were then reared on clean uninfested cowpea

seeds (Ife brown variety) and served as the stock culture of the insects used for the insect bioassay. The insects were reared under a laboratory condition of 28 ± 2 °C temperature and 75 ± 5% relative humidity, photoperiod of 12 h light followed by 12 h dark (12L:12D).

Identification and external sex differences of adult C. maculatus (Fab.)

The identification and sexing of *C. maculatus* were carried out according to Halstead (1963), Odeyemi and Daramola (2000). Males have comparatively shorter abdomen, and the terminal segment's dorsal side is sharply curved downward and inward. In contrast, the females have comparatively longer abdomen and the dorsal side of the terminal segment is only slightly deflexed downward. The females also have two visible dark spots on their elytra while the markings or the visible dark sports in the males are less distinct (Halstead 1963; Odeyemi and Daramola 2000). Females are larger than males (Halstead 1963; Odeyemi and Daramola 2000).

Collection and preparation of plant powders

Fresh leaves of *C. capitatum, P. fraternus* and *T. diversifolia* (Table 1) used for the study were collected from farmland in Iju (7°23′39.75 N, 5°15′32.78E), Akure North Local Government Area, Ondo State, Nigeria. The leaves were first rinsed in clean water and air-dried for one month. After air drying, the leaves were separately pulverized into fine powders using an electric blender, JTC Omni Blender V$^{(R)}$ (Model TM-800). The fine powders were sieved through a nylon mesh (1 mm^2). The powders were placed in air tight containers and labelled separately and stored at 4 °C in a refrigerator to maintain their quality.

Preparation of methanolic extract of experimental plants

About 400 g each of *C. capitatum, P. fraternus* and *T. diversifolia* powder were soaked in an extraction bottle containing 800 ml each of absolute met hanol for 72 h (Udo 2011; Ileke et al. 2020; 2021a; b). The mixture was stirred intermittently using a glass rod in order to ensure homogeneity in extraction (Udo 2011). The solvent and extracts were separated using a rotary evaporator at 30 to 40 °C with the rotary speed of 3 to 6 rpm for 8 h (Udo 2011). The resulting extracts were then air-dried to remove traces of the solvent (Udo 2011). The extracts were kept in labelled plastic bottles.

Table 1 List of plants used

Family name	Scientific name	Common name	Parts used
Lamiaceae	*Clerodendrum capitatum*	Bag-flower	Leaf
Phyllanthaceae	*Phyllanthus fraternus*	Gulf Leaf-flower	Leaf
Asteraceae	*Tithonia diversifolia*	Tree Marigolds	Leaf

Phytochemical screening of the leaves of the experimental plants

Chemical tests were carried out on the powders and methanolic extracts of the leaves of *C. capitatum, P. fraternus* and *T. diversifolia* for the qualitative determination of phytochemical constituents using standard procedures as described by Harborne (1973), Trease and Evans (1985) and Sofowora (1993).

Insect bioassay

Toxicity of plant leaf powders to adult C. maculatus

Clean, undamaged and unifested cowpea seeds of Ife brown variety (20 g) was introduced into 250 ml of plastic cups. After that, different dosages (0.5, 1.0, 2.0, 4.0 and 5.0 g/w/w) of pulverized powders each of *C. capitatum, P. fraternus* and *T. diversifolia* were weighed and admixed separately with 20 g of cowpea seeds inside a 250 ml transparent plastic cups separately. The mixtures were shaken adequately to ensure the proper coating of the seeds with the powder. Ten (10) pairs of newly emerged adult *C. maculatus* (less than 2 days old) were put into each of the transparent plastic cups containing the treated seeds. Treatments were replicated four times. The control treatment only involved 20 g of clean un-infested cowpea seeds with ten (10) copulating pairs. The plastic cups were then covered with muslin cloth held tightly with rubber band to allow aeration and at the same time prevent the escape of insects. Insect mortality was observed daily for 5 days (120 h). Adult beetles were considered dead when they did not show signs of movement or response to gentle pin probing. Oviposition on treated and untreated seeds was determined by counting the total number of eggs laid on each seed. The insect bioassay was kept inside the insect rearing cage and daily observations were made until the first filial generation adult emergence. The newly emerged adults were counted, recorded and removed on a daily basis until there was no more adult emergence for five consecutive days. All the data on the percentage of adult mortality was corrected using Abbott formula (1925). Thus:

$$P_T = \frac{P_o - P_c}{100 - P_o} \times \frac{100}{1} \quad (1)$$

where P_T = corrected mortality (%); P_O = observed mortality (%); P_C = control mortality (%).

Also, the percentage adult emergence was calculated using the method of Odeyemi and Daramola (2000).

$$\% \text{ Adult emergence} = \frac{\text{Total number of adult emerged}}{\text{Total number of eggs laid}} \text{ x } \frac{100}{1} \quad (2)$$

Effect of methanolic extracts of experimental plants on the mortality of adult C. maculatus

An aliquot of 0.1, 0.2, 0.5, 1.0 and 2.0 ml of the extracts of *C. capitatum, P. franternus* and *T. diversifolia* were measured using a 2 ml graduated syringe and admixed separately with 20 g of clean, undamaged and un-infested cowpea seeds inside a 250 ml transparent plastic cups. Ten (10) pairs newly emerged adult *C. maculatus* (less than 2 days old) were put into each of the transparent plastic cups containing the treated seeds. Treatments were replicated four times. The control experiment only contained 20 g of clean un-infested cowpea seeds with the same equal number of and ratio of adult *C. maculatus*. The plastic cups were then covered with muslin cloth held tightly with rubber band to allow aeration and prevent the escape of insects. Insect mortality was observed daily for 5 days (120 h). Adult beetles were considered dead when they did not show signs of movement or response to gentle pin probing. The total number of eggs laid was taken and recorded as was determined earlier above The insect bioassay was done inside the insect rearing cage and daily observations were made until first filial generation adult emergence. The newly emerged adults were counted, recorded and removed on a daily basis until there was no more adult emergence for five consecutive days.

Statistical analysis

Data collected from the laboratory tests were subjected to analysis of variance (ANOVA) at 5% significance level and treatment means were separated using Tukey's Test. Log-Probit model analysis was carried out on percentage mortality of the adult *C. maculatus* to determine the 50% lethal dose/concentration (LD_{50}/LC_{50}) and 90% lethal dose/concentration (LD_{90}/LC_{90}) (Finney 1971).

Results

Phytochemicals screening of C. capitatum, P. fraternus and T. diversifolia

Phytochemicals present in powders and methanolic extracts of *C. capitatum, P. fraternus* and *T. diversifolia* leaves are furnished in Table 2. The results of qualitative analysis showed that alkaloid, tannin, saponin, flavonoids and cardiac glycosides were present in powder and methanolic extracts of all the experimental plants.

Contact Toxicity of C. capitatum, P. fraternus and T. diversifolia powders on adult mortality of C. maculatus

Contact toxicity of *C. capitatum, P. fraternus* and *T. diversifolia* powders on adult mortality of *C. maculatus* is presented in Table 3. Plants powders at tested concentration showed that beetle mortality ranged from 32.5 to 100% after 24 h of treatment. *Tithonia diversifolia* powders was the most potent plant powder to cowpea beetle. It evoked 76.5, 92.5, 100.0, 100.0% and 100% mortality of adult insect at concentrations of 05 g, 1.0 g, 2.0 g, 4.0 g and 5.0 g/20 g of cowpea seeds after 5 days of exposure, respectively. This was followed by *P. fraternus* that evoked 62.5, 75.0, 90.0, 100.0% and 100.0% of adult mortality of *C. maculatus* after 120 h of post-treatment at concentration of 0.5 g, 1.0 g, 2.0 g, 4.0 g and 5.0 g/20 g of cowpea seeds, respectively. *Clerodendrum capitatum* powder was the least toxic plant powder to *C.*

Table 2 Qualitative analysis of Phytochemicals in experimental plants

Phytochemicals	*C. capitatum*		*P. fraternus*		*T. diversifolia*	
	Powder	Methanolic extract	Powder	Methanolic extract	Powder	Methanolic extract
Alkaloids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+
Phlobatannins	–	–	–	–	–	–
Anthraquinones	–	–	–	–	–	–

Keys: + present; −Absent

Table 3 Effects of *C. capitatum, P. fraternus* and *T. diversifolia* powders to adult *C. maculatus*

Plant Powders	Conc. (g)	Mean Mortality ± S.E. (%) in Hours				
		24	48	72	96	120
T. diversifolia	0.5	55.00 ± 2.89^{d}	60.00 ± 2.75^{de}	65.00 ± 2.89^{cd}	72.50 ± 2.50^{c}	$77.5\ 0 \pm 2.25^{b}$
	1.0	60.00 ± 2.75^{e}	65.00 ± 2.89^{de}	72.50 ± 2.50^{de}	80.00 ± 2.75^{c}	92.50 ± 2.50^{cd}
	2.0	67.50 ± 2.25^{ef}	77.50 ± 2.25^{ef}	80.00 ± 2.75^{e}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}
	4.0	100.00 ± 0.00^{g}	100.00 ± 0.00^{g}	100.00 ± 0.00^{f}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}
	5.0	100.00 ± 0.00^{g}	100.00 ± 0.00^{f}	100.00 ± 0.00^{f}	100.00 ± 0.00^{e}	100.00 ± 0.00^{d}
P. fraternus	0.5	50.00 ± 2.75^{cd}	57.50 ± 2.25^{cd}	62.50 ± 2.89^{c}	67.50 ± 2.25^{bc}	70.00 ± 2.75^{b}
	1.0	55.00 ± 2.89^{cd}	60.00 ± 2.75^{de}	70.00 ± 2.75^{de}	77.50 ± 2.25^{c}	87.50 ± 2.25^{cd}
	2.0	62.50 ± 2.50^{e}	70.00 ± 2.50^{e}	77.50 ± 2.25^{e}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}
	4.0	90.00 ± 2.75^{g}	100.00 ± 0.00^{g}	100.00 ± 0.00^{f}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}
	5.0	100.00 ± 0.00^{g}	100.00 ± 0.00^{g}	100.00 ± 0.00^{f}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}
Clerodendrum capitatum	0.5	32.50 ± 2.89^{b}	40.00 ± 2.75^{b}	47.50 ± 2.25^{b}	55.00 ± 2.89^{b}	62.50 ± 2.89^{b}
	1.0	30.00 ± 2.75^{b}	45.00 ± 2.89^{bc}	55.00 ± 2.89^{bc}	67.50 ± 2.25^{bc}	75.00 ± 2.89^{bc}
	2.0	40.00 ± 2.75^{bc}	47.50 ± 2.25^{bc}	60.00 ± 2.75^{cd}	75.00 ± 2.89^{c}	90.00 ± 2.75^{d}
	4.0	57.50 ± 2.25^{de}	67.50 ± 2.25^{ef}	85.00 ± 2.89^{e}	90.00 ± 2.75^{d}	100.00 ± 0.00^{d}
	5.0	77.50 ± 2.25^{f}	90.00 ± 2.75^{f}	100.00 ± 0.00^{f}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}
Control	0.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is a mean ± standard error of four replicates. Means followed by the same letter among the treatments are not significantly different ($p > 0.05$) using Tukey's Test

maculatus. It caused 62.5%, 75.0%, 90.0%, 100.0% and 100% mortality of adult insect at concentrations of 05 g, 1.0 g, 2.0 g, 4.0 g and 5.0 g/20 g of cowpea seeds after 5 days of exposure, respectively. There was no significant difference ($p > 0.05$) in the tested plant powders at concentrations of 0.2 g, 4.0 g and 5.0 g compared with the untreated seeds.

Lethal dose (LD) of C. capitatum, P. fraternus and T. diversifolia powders against adult C. maculatus

The lethal doses of different plant powders against adult *C. maculatus* are given in Table 4. The required dosage calculated to cause 50% (LD_{50}) and 90% (LD_{90}) insect mortality after 24 h were 2.06 and 31.18 g; 0.68 and 4.24 g; and 0.51

Table 4 Lethal Dose (LD) of *C. capitatum, P. fraternus* and *T. diversifolia* powders against adult *C. maculatus*

Plant parts	Exposure period (hours)	LD_{50} (LCL-UCL) (g)	LD_{90} (LCL-UCL) (g)	χ^2	P-value
C capitatum	24	2.06 (0.98–4.30)	31.18 (14.92–65.17)	14.98	0.00
	42	1.16 (0.60–2.24)	13.33 (6.91–25.72)	20.84	0.00
	72	0.71 (0.34–1.52)	5.03 (4.72–15.43)	25.90	0.00
	96	0.43 (0.20–0.91)	3.31 (2.30–10.23)	11.94	0.01
	120	0.34 (0.17–0.65)	2.13 (1.11–4.10)	6.64	0.08
P. fraternus	24	0.68 (0.35–1.31)	4.24 (3.38–12.63)	25.99	0.00
	42	0.28 (0.06–1.19)	2.77 (1.52–7.84)	34.53	0.00
	72	0.18 (0.05–0.63)	2.23 (1.79–2.71)	22.46	0.00
	96	0.18 (0.07–0.47)	1.40 (1.15–2.62)	11.96	0.01
	120	0.16 (0.15–0.52)	1.34 (0.63–2.14)	3.36	0.00
T. diversifolia	24	0.51 (0.07–1.42)	3.19 (2.75–4.17)	36.77	0.00
	42	0.26 (0.10–0.80)	2.33 (2.10–5.12)	22.77	0.00
	72	0.16 (0.05–0.52)	2.05 (1.53–2.49)	19.44	0.00
	96	0.12 (0.07–0.41)	1.39 (1.03–2.31)	11.58	0.01
	120	0.09 (0.03–0.34)	1.30 (0.47–2.14)	1.90	0.59

$\chi 2$ = Chi-square value, *LCL* Lower confidence limit and *UCL* Upper confidence limit

and 3.19 g for *C. capitatum, P. fraternus* and *T. diversifolia* powders, respectively. These values were observed to reduce as the period of exposure increased. From the calculations, *T. diversifolia* was observed to have the lowest lethal dose, followed by *P. fraternus* while *C. capitatum* has the highest lethal dose across all the periods of exposure.

Contact toxicity of C. capitatum, P. fraternus and T. diversifolia powders on oviposition and Adult emergence of C. maculatus

The effects of powders of *C. capitatum, P. fraternus* and *T. diversifolia* on oviposition and adult emergence of cowpea beetle, *C. maculatus* is presented in Table 5. The number of eggs laid by beetle on treated cowpea seeds was significantly lower ($p < 0.05$) than untreated seeds (control). There was no significant difference ($p > 0.05$) in the mean number of eggs laid on the treated seeds with *C. capitatum, P. fraternus* and *T. diversifolia* powders at concentration 2, 4 and 5 g/20 g of cowpea seeds. On *C. capitatum* powders, the numbers of egg laid were 20, 12, 6, 1.5 and 0.0 at concentrations 0.5, 1.0, 2.0, 4.0 and 5.0 g/20 g of cowpea seeds, respectively. Similarly, *P. fraternus* powders significantly reduced the number of egg laid at dosage rates 4 and 5 g/20 g seed. The oviposition and % adult emergence in the untreated cowpea seeds was significantly different ($p < 0.05$) from oviposition and emergence in the treated cowpea seeds. There was no sign of egg laying and adult emergence in the cowpea seeds treated with 2, 4 and 5 g of *T. diversifolia* powder.

Table 5 Contact toxicity of *C. capitatum, P. fraternus* and *T. diversifolia* powders on oviposition and adult emergence of *C. maculatus*

Plant powders	Conc. (g)	Mean Oviposition	% adult emergence
C. capitatum	0.5	20.00 ± 1.18^{b}	10.00 ± 1.03^{a}
	1.0	12.00 ± 1.05^{b}	0.00 ± 0.00^{a}
	2.0	6.00 ± 0.04^{a}	0.00 ± 0.00^{a}
	4.0	1.50 ± 0.03^{a}	0.00 ± 0.00^{a}
	5.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
P. fraternus	0.5	12.50 ± 1.02^{b}	8.00 ± 1.08^{a}
	1.0	7.00 ± 0.04^{a}	0.00 ± 0.00^{a}
	2.0	5.50 ± 0.08^{a}	0.00 ± 0.00^{a}
	4.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	5.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
T. diversifolia	0.5	5.00 ± 0.04^{a}	0.00 ± 0.00^{a}
	1.0	3.50 ± 0.06^{a}	0.00 ± 0.00^{a}
	2.0	1.00 ± 0.01^{a}	0.00 ± 0.00^{a}
	4.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	5.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Control	0.0	53.50 ± 2.78^{b}	56.07 ± 2.79^{d}

Each value is a mean ± standard error of four replicates. Means followed by the same letter among the treatments are not significantly different ($p > 0.05$) using Tukey's Test

Contact toxicity of C. capitatum, P. fraternus and T. diversifolia methanolic extracts on adult mortality of C. maculatus

Contact toxicity of *C. capitatum, P. fraternus* and *T. diversifolia* extracts on adult mortality of *C. maculatus* is presented in Table 6. Plants extracts at tested concentrations had mortality ranged from 50 to 100%. There was no significant difference ($p > 0.05$) among the tested plant methanolic extracts at concentrations 1.0 and 2.0 mls when compared with untreated seeds after 24 h of post-treatment. At 24 h, *T. diversifolia* extracts caused 60.0, 75.0, 82.5, 100.0 and 100.0% adult mortality of cowpea beetle at concentrations of 0.1, 0.2, 0.5, 1.0 and 2.0 ml/20 g v/w of cowpea seeds, respectively. Similarly, *P. fraternus* evoked 57.5, 67.5, 77.5, 95.0 and 100.0% mortality of adult *C. maculatus* at concentration of 0.1, 0.2, 0.5, 1.0 and 2.0 ml/20 g of cowpea seeds, respectively. *Tithonia diversifolia* extract was the most potent and it caused 100% mortality of adult *C. maculatus* at all tested concentrations (0.1, 0.2, 0.5, 1.0 and 2.0 ml) after 5 days of exposure. Methanolic extract of *P. fraternus* caused 95.0, 100.0, 100.0, 100.0 and 100.0% of adult mortality of *C. maculatus* after 5 days of post-treatment with 0.1, 0.2, 0.5, 1.0 and 2.0 ml/20 g of cowpea seeds, respectively. *Clerodendrum capitatum* methanolic extract was the least toxic causing 80, 97.5, 100, 100% and 100% of adult mortality of beetle at concentrations of 0.1, 0.2, 0.5, 1.0 and 2.0 ml/20 g of cowpea seeds, respectively.

Lethal concentration (LC) of C. capitatum, P. fraternus and T. diversifolia powders against adult C. maculatus

The lethal concentration of different plant powders against adult *C. maculatus* are given in Table 7. The required concentrations calculated to cause 50% (LC_{50}) and 90% (LC_{90}) insect mortality after 24 h was 0.10 and 0.77 ml; 0.09 and 0.70 ml; and 0.05 and 0.68 ml for *C. capitatum, P. fraternus* and *T. diversifolia* extracts, respectively. These values were observed to reduce as the period of exposure increased. From the calculations, *T. diversifolia* was observed to have the lowest lethal dose while *C. capitatum* the highest across the periods of exposure. However, some values could not be calculated because no optimal solution was found.

Contact toxicity of C. capitatum, P. fraternus and T. diversifolia extracts on oviposition and adult emergence of C. maculatus

Effects of *C. capitatum, P. fraternus* and *T. diversifolia* extracts on oviposition and adult emergence of cowpea beetle, *C. maculatus,* is presented in Table 8. The number of eggs laid by beetle on treated cowpea seeds was

Table 6 Effects of *C. capitatum, P. fraternus* and *T. diversifolia* extracts to adult *C. maculatus*

Plant Extracts	Conc.(ml)	Mean Mortality ± S.E. (%) in Hours				
		24	48	72	96	120
C. capitatum	0.1	50.00 ± 2.75^{b}	60.00 ± 2.75^{b}	67.50 ± 2.25^{b}	75.00 ± 2.89^{b}	80.00 ± 2.75^{b}
	0.2	62.50 ± 2.50^{bc}	70.00 ± 2.75^{bc}	80.00 ± 2.75^{c}	87.50 ± 2.25^{bc}	97.50 ± 0.25^{c}
	0.5	70.00 ± 2.75^{cd}	77.50 ± 2.25^{cd}	87.50 ± 2.50^{c}	100.00 ± 0.00^{d}	100.00 ± 0.00^{c}
	1.0	100.00 ± 0.00^{f}	100.00 ± 0.00^{f}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{c}
	2.0	100.00 ± 0.00^{f}	100.00 ± 0.00^{f}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{c}
P. fraternus	0.1	57.50 ± 2.25^{bc}	$70.00 \pm 2.75b^{c}$	80.00 ± 2.75^{c}	87.50 ± 2.25^{bc}	95.00 ± 2.89^{c}
	0.2	67.50 ± 2.25^{cd}	75.00 ± 2.89^{cd}	85.00 ± 2.89^{c}	92.50 ± 2.50^{cd}	100.00 ± 0.00^{c}
	0.5	77.50 ± 2.25^{d}	87.50 ± 2.25^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{c}
	1.0	95.00 ± 2.89^{f}	100.00 ± 0.00^{f}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{c}
	2.0	100.00 ± 0.00^{f}	100.00 ± 0.00^{f}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{c}
T. diversifolia	0.1	60.00 ± 2.75 cd	82.50 ± 2.50^{de}	90.00 ± 2.75^{d}	97.50 ± 2.50^{d}	100.00 ± 0.00^{c}
	0.2	75.00 ± 2.75^{de}	87.50 ± 2.25^{e}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{c}
	0.5	82.50 ± 2.50^{e}	100.00 ± 0.00^{f}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{c}
	1.0	100.00 ± 0.00^{f}	100.00 ± 0.00^{f}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{c}
	2.0	100.00 ± 0.00^{f}	100.00 ± 0.00^{f}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{c}
Control	0.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is a mean ± standard error of four replicates. Means followed by the same letter among the treatments are not significantly different ($p > 0.05$) using Tukey's Test

Table 7 Lethal Concentration (LC) of *C. capitatum, P. fraternus* and *T. diversifolia* powders against adult *C. maculatus*

Plant parts	Exposure period (hours)	LC_{50} (LCL-UCL) (ml)	LC_{90} (LCL-UCL) (ml)	χ^2	*P*-value
Clerodendrum capitatum	24	0.10 (0.03–0.29)	0.77 (0.66–1.07)	27.37	0.00
	48	0.09 (0.01–0.15)	0.56 (0.46– 0.94)	20.05	0.00
	72	0.06 (0.01–0.12)	0.37 (0.20–0.63)	8.70	0.03
	96	*	*	*	*
	120	*	*	*	*
P. fraternus	24	0.09 (0.04–0.18)	0.70 (0.39–1.66)	9.88	0.02
	48	0.06 (0.01–0.09)	0.31 (0.25–1.02)	10.33	0.00
	72	*	*	*	*
	96	*	*	*	*
	120	*	*	*	*
T. diversifolia	24	0.05 (0.02–0.13)	0.68 (0.39–2.87)	13.16	0.00
	48	*	*	*	*
	72	*	*	*	*
	96	*	*	*	*
	120	*	*	*	*

χ^2 = Chi-square value, *LCL* Lower confidence limit and UCL = Upper confidence limit

* = Values not calculated because no optimal solution was found

significantly lower ($p < 0.05$) than untreated seeds. There was no significant difference ($p > 0.05$) among the mean number of eggs laid on seeds treated with *C. capitatum, P. fraternus* and *T. diversifolia* extracts at all tested concentrations apart from 0.1 ml/20 g of cowpea seeds. On seeds treated with *C. capitatum* extract, the numbers of egg laid were 7.5, 2.0, 0.0, 0.0 and 0.0 at concentrations of 0.1, 0.2, 0.5, 1.0 and 2.0 ml/20 g of cowpea seeds, respectively. Similarly, *P. fraternus* extract significantly reduced the number of egg laid at concentrations of 0.5, 1.0 and 2.0 ml. The oviposition and percentage adult emergence in the untreated seeds was significantly different ($p < 0.05$) from oviposition and emergence in the treated cowpea seeds. There was no sign of egg laying and adult

Table 8 Contact toxicity of *C. capitatum, P. fraternus* and *T. diversifolia* extracts on oviposition and Adult Emergence of *Callosobruchus maculatus*

Plant powders	Conc. (ml)	Mean Oviposition	% adult emergence
C. capitatum	0.1	7.50 ± 0.03^{b}	13.50 ± 1.25^{b}
	0.2	2.00 ± 0.01^{ab}	0.00 ± 0.00^{a}
	0.5	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	1.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	2.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
P. fraternus	0.1	5.00 ± 0.02^{b}	0.00 ± 0.00^{a}
	0.2	1.00 ± 0.01^{ab}	0.00 ± 0.00^{a}
	0.5	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	1.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	2.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
T. diversifolia	0.1	5.00 ± 0.04^{b}	0.00 ± 0.00^{a}
	0.2	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	0.5	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	1.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	2.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Control	0.0	53.50 ± 2.78^{b}	56.07 ± 2.79^{d}

Each value is a mean ± standard error of four replicates. Means followed by the same letter among the treatments are not significantly different ($p > 0.05$) using Tukey's Test

emergence in the maize seeds treated with 0.1, 0.2, 0.5, 1.0 and 2.0 ml of *P. fraternus* and *T. diversifolia* extracts.

Discussion

Entomologists worldwide have employed many procedures to screen plant materials for their efficacies against cowpea beetle, *C. maculatus* (Adedire and Lajide 1999; Ogunwolu and Odunlami 1996; Okonkwo and Okoye 1996; Akinkurolere 2012; Ileke 2014; Ileke et al. 2020b). In all the tested procedures, the botanicals have proven effective in reducing damage caused by this notorious stored product beetle through contact toxicity, antifeedant, fumigant, ovicidal and larvicidal properties (Ogunwolu and Odunlami 1996; Boeke et al. 2001; Akinkurolere et al. 2006; Akinkurolere 2012). Many of these botanicals that possessed insecticidal properties are well distributed in the world's tropical zones (Akinkurolere et al. 2006).

The results obtained from this research has shown that all the plant powders and extracts tested for insecticidal activities were effective in suppressing the population of *C. maculatus* when compared with the control experiment. It has been established with the data of this research work that the percentage beetle mortality is directly proportional to dosage rate and period of exposure. The present research also showed that the plant extracts were more toxic to the beetles than their plant powders. Similar observation was reported by Ileke (2019) on the effectiveness of powders and extracts of *Alstonia boonei* part in the management of cowpea beetle. Ileke et al. (2020a) also made similar comments on the utilization of *Acanthus montanus*, *Argyreia nervosa*, *Alchornea laxiflora* and *Acanthospermum hispidum* as protectant of maize grains against *S. zeamais*. Asawalam et al. (2007) reported that insecticidal activity of any plant material depends on the active ingredients present in the extract. Lale (1995) also reported that plant extracts have a great affinity for lipids and could penetrate the cuticle of insects. The leaf powder and extract of *T. diversifolia* at all the dosages tested were the most potent against *C. maculatus*. This was followed by *P. fraternus*, while *C. capitatum* was moderately effective. Adesina et al. (2016) reported the effectiveness of *C. capitatum* in the management of the hide beetle, *Dermestes maculatus* DeGeer infesting smoked catfish. The observed lethal effects of *T. diversifolia* could be linked to its strong choky odour, which evoked suffocating action on the beetle. This result validated the report of Adedire and Akinneye (2004) who reported that the powder of *T. diversifolia* applied at 3–6% evoked 63–75% *C. maculatus* mortality. Adoyo et al. (1997) also found *T. diversifolia* effective in an on-farm control of termites in the Busia district of Kenya. The insecticidal activity of the tree marigold could be ascribed to the presence of two sesquiterpene lactones, seven germacranolides and four eudesmanolides which were isolated from the aerial parts of *T. rotundifolia* by Bohlmann et al. (1981). Kou and Lin (1999) also isolated a novel dinorxanthane sesquiterpene called diversifolide [4,15-dinor-3-hydroxy-1 (5)-xanthen- 12,8-olide], a new chromone and four other known compounds from the root of *T. diversifolia.* Some of these compounds are believed to be responsible for its allelopathic and insecticidal activities (Adedire and Akinneye 2004).

The entomocidal potential of the powder and oil extract of *P. fraternus* were revealed in this study. It was the second most toxic plant to cowpea beetle in this research work in terms of effectiveness, it was the second most toxic plant to cowpea beetle in this research work. This means that the leaf powder and methanolic extract were poisonous to adult *C. maculatus* and could serve as a bioinsecticide. This result agrees with the findings of Adesina et al. (2014) who reported that the leaf powder of *P. fraternus* was effective in suppressing the infestation of *Dermestes maculatus* on smoked-dried fish. The study indicated that the higher dosage level of powder and methanolic extract were the most effective in the application rates compared to the untreated control.

The insecticidal activity of *P. fraternus* may be attributed to the presence of biochemical constituents present in the plant. Rastogi and Mahrotra (1990) reported the chemical constituents of *P. fraternus* to include phyllanthin, hypophyllanthin, niranthin, nirtetralin, phyltetratralin,

kaempferol- 4- rhamnopyranoside and erio dictylol-7-rhamnopyranoside etc. These bioactive agents could possess, among other pharmaceutical properties, a depolarizing neuromuscular blocking action which could result to the death of insect (Udoh et al. 1999). Various researchers have also reported that plant products disrupt the process of gaseous exchange in insects (Adedire et al. 2011; Ojo and Ogunleye 2013; Ileke et al. 2014). Therefore, the lethal effects of these plant products on *C. maculatus* could be due to contact toxicity. The trachea, which is the respiratory organ of insects and normally opens at the surface through spiracles, might have been blocked by these powders and extracts thereby leading to difficulty in breathing which eventually led to suffocation and death (Adedire et al. 2011).

The experimental plants significantly reduced the number of eggs laid by gravid female *Callosobruchus maculatus*. The ability of plant powders and oil extracts to cause a reduction or complete inhibition of oviposition by female insect pests of order Coleoptera has been reported by many researchers (Adedire and Akinneye 2004; Ileke and Oni 2011; Akinkurolere 2012; Obembe and Kayode 2013; Ileke et al. 2020a, b; 2021a,b). The results obtained on oviposition and adult emergence showed that the plant powders could serve as alternative methods to reduce the population of *C. maculatus* on stored seeds. The observed reduction in the number of eggs laid by the insect in this study could be linked with respiratory impairment, which probably affected metabolic activities and consequently other systems of the beetles' body (Obembe and Kayode 2013; Ileke et al. 2014; Obembe and Ojo 2018; Ojo et al. 2018). The high percentage of insect mortality recorded in the treated seeds especially the seeds treated with the leaf powder and methanolic extract of *T. diversifolia* could be responsible for the low number of eggs laid by the beetle. The few eggs that were laid on the treated seeds were unable to glue to the surface of the seeds due to the presence of the plant products. This must have accounted for the mortality of the eggs and hence the zero adult emergence (Ojo and Ogunleye 2013). Adedire and Lajide (2001) observed that adult emergence could be reduced when the eggs and larvae are in close contact with the plant powders and oil extracts thus causing oviposition deterrence. The calculated lethal dose (LD_{50} and $_{90}$) and concentrations (LC_{50} and $_{90}$) of the plant powders and extracts showed that *T. diversifolia* had the lowest values, while *Clerodendrum capitatum* the highest across all period of exposure.

The phytochemicals present in the powder and methanolic extracts of the leaf of *C. capitatum, P. fraternus* and *T. diversifolia* are alkaloids, saponins, tannin and cardiac glycosides with flavonoids found in both the aqueous and methanolic extracts of *P. fraternus*. In traditional usage, decoction or infusions of herbs are usually made with either alcohol or water as the solvent. Fernado et al. (2005) reported that most plants are known to possess chemical substances like terpenoids, saponins, tannins, flavonoids and alkaloids among others which have been found to be toxic to insect pests. The toxicity and antifeedant effects of alkaloids on stored products insect have been reported (Yang et al. 2006). These observed mortality properties of the three botanicals could be linked to volatile constituents such as, flavonoid, tannins, saponins and alkaloids present in the plants (Ileke et al. 2014).

Conclusion

The present research work investigated the effect of *C. capitatum, P. fraternus* and *T. diversifolia* powder and extract against cowpea beetle, *C. maculatus*. *T. diversifolia* was found to be the most effective among the plants tested for insecticidal activity against *C. maculatus*. This plant could be a better alternative to synthetic and conventional insecticides since it is abundant in our location. *P. fraternus* powder and extracts were also effective as seed protectant against cowpea bruchid, *C. maculatus*. The results obtained in this research work suggested that *C. capitatum, P. fraternus* and *T. diversifolia* seed powder and extract could be used as biopesticides against cowpea beetle.

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Declarations

Coflict of interest The authors declare that they have no competing interests.

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