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Efficacy of extracts of *Tithonia diversifolia* (Hemsl.) A. Gray as protectant against maize weevil (*Sitophilus zeamais* [Motsch.]) and cowpea weevil, *Callosobruchus maculatus* F. on stored grains

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Abstract

This study sought to determine the efficacy of *Tithonia diversifolia* (Mexican Sunflower) as pesticides against maize weevil *Sitophilus zeamais* and cowpea weevil *Callosobruchus maculatus.* The research was conducted to ascertain the toxicity potency of *T. diversifolia* on insects' mortality, repellency, adult emergence, seed damage and weight loss for the treatment of stored products and determine their minimum effective dosage for application. *T. diversifolia* collected from the premises of Kwara State University, Malete were air-dried, ground and dissolved in 50 g to 600 ml of four different extracts (Ethanol, Methanol, n-Hexane and aqueous). *S. zeamais* and *C. maculatus* were reared in the laboratory at 60-75% relative humidity and 25-35 ºC. After the whole extraction, ten unsexed *S. zeamais* and *C. maculatus* were infested in 10 g of grains and replicated three times in each application of extracts with different treatment levels. The mortality and other parameters were counted post-treatment. The mean results of the parameters studied were significantly different at ($P<0.05$). N-hexane extracts was most toxic to *S. zeamais* while Methanol extract was most toxic to *C. maculatus.* Thus, all the extracts were potent, and they reduced insects' oviposition, increased mortality, improved repellence, inhibited reproduction, grain damage and weight loss in storage compared to the untreated control. Subjected to the qualitative phytochemical screening, active compounds discovered to be present in *T. diversifolia* include tannin, flavonoid, saponin, phenol, terpenoid, glucosides and alkaloid. The study proffers the use of *T. diversifolia,* a readily available, safe and ecofriendly biopesticide for farmers as short and long-term storage of the stored produce.

Keywords: *Sitophilus zeamais*, *Callosobruchus maculatus, Tithonia. diversifolia,* efficacy, maize, cowpea.

INTRODUCTION

Storage of food is mostly important to food security in developed countries and it has been a poised challenge in

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developing countries most especially controlling stored pest, although there are synthetic chemicals which are readily available for use (Sola *et al*., 2014). Storage is crucial act which reinforces marketing efficiency by providing utility (Adejumo and Raji, 2007). Due to the seasonality of crop production in many African nations, in which there is only one rainy season annually, effective maize storage systems are imperative (Owusu *et al.,* 2008). Insecticides are effectively used against postharvest insect pests but are often faced with variety of drawbacks (Mulungu *et al*., 2010).

The maize weevil, *Sitophilus zeamais* among other insect pests of cereals is a serious pest of maize. In Nigeria, the postharvest losses due to *S. zeamais* have been recognized as a significant problem, with grain losses ranging from 20 to 90% being reported for stored untreated maize (Dhliwayo and Pixley, 2003). Maize is exposed to this insect pest attack on the field, during harvest and in storage (Munjoma, 2004). On the other hand, the cowpea weevil, *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae: Bruchinae) is also a serious pest of stored cowpea grains in sub-Saharan Africa (Bekele *et al*., 1997, Ojo *et al*., 2016). Post-harvest losses of cowpea as high as 60% in storage caused by *C. maculatus* infestation have been reported in different parts of Nigeria and other West African Countries (Dabire *et al*., 2005). *C. maculatus* is very destructive on account of its short life cycle. The infestation by this beetle cause tremendous reduction in quality and quantity, viability and marketability of cowpea seeds (DeBoer *et al*., 2003). One alternative control method is the use of plant extracts (Gallo *et al.,* 2002; Gotyal *et al*., 2010) which favours natural enemies, necessary for biological balance (Hole *et al.,* 2015).

A few plant substances have been considered for use as insect repellents and anti-feedants for example essential oil derived from orange peels has been shown to be toxic on *C. maculatus*. *Cymbopogon citratus* essential oil has also been reported to be toxic on *C. maculatus* larval and adult (de Souza Alves *et al*., 2019), as well as maize weevil *S, zeamais*. Some of the botanicals that has also been used include: garlic, *Allium sativum* L. chili pepper, *Capsicum* spp. Siam weed, *Chromolaena odorata* (L.) moringa, *Moringa oleifera* Lam. tobacco, *Nicotiana tabacum* L. clove basil, *Ocimum gratissimum* L. tree marigold, *Tithonia diversifolia* (Hemsley) and bitter leaf, *Vernonia amygdalina* (Delile) (Asterales: Asteraceae) (Ojo *et al*., 2016).

Tithonia diversifolia of the family: Asteraceae is a common shrub on field boundaries, grassland and disturbed land commonly called 'Mexican sunflower'. Olayinka *et al*. (2015) reported the presence of alkaloids, tannins, flavonoids, saponins, terpenoids and phenols in the leaves, roots and stems of *T. diversifolia*. Likewise, glycosides were detected in appreciable proportions in aqueous and methanolic extracts of shoots of *T. diversifolia* (Otusanya and Ilori, 2012). This plant is employed by farmers as a bio-pesticide to replace hazardous and expensive synthetic pesticides (Pavela *et al*., 2018). Due to the fact that stored maize and cowpea are vulnerable to the attack of *S. zeamais* and *C. maculatus*, respectively causing economic damage if not properly controlled, and with the deleterious attribute of synthetic insecticide to the ecosystem, this study seeks to investigate the potency of *T. diversifolia* as a biopesticide. Therefore, the main objective of this experiment was to evaluate the efficacy of *Tithonia diversifolia* extracts as biopesticide against *S. zeamais* and *C. maculatus* in the store.

MATERIALS AND METHODS

Insects Rearing and Collection of Clean Maize and Cowpea

Two hundred unsexed adults *Sitophilus zeamais* and *Callosobruchus maculatus* were introduced into glass jars with mesh lid containing 1 kilogram of maize and cowpea samples respectively and were kept at ambient temperature of 27 ± 3°C and relative humidity of 65 ± 5% in the laboratory. The insects were left on the grains to feed and lay eggs for 14 days and thereafter removed. The jars were left undisturbed until emergent of new progenies. The emergent progenies were used as insect stock culture for bioassay in the study. The clean uninfested maize grains – variety DMRESR and cowpea seeds – IAR 48 variety used for this study were purchased from Premier Seed Company® in Ilorin, Kwara State, Nigeria. The grains and seeds were cold-shocked at 10°C for 12 days to kill all incipient infestations on the grains.

Collection and Preparation of Plant Materials

Tithonia diversifolia (Mexican sunflower) whole plants were collected from the premises of Kwara State University, Malete, Nigeria. The plant was identified at the Plant Biology Unit of Kwara State University, Malete, Nigeria. The collected sample was rinsed with clean water and air dried separately in the laboratory for three (3) weeks. The dried leaf of the plant was ground into powder using Marlex electrolyne excella® universal grinder, weighed and kept separately in an airtight polyethylene bag. The powder was used for extraction and the bioassays.

Preparation of extracts of the plant

The four solvents used for the extraction were Ethanol, n-Hexane, Methanol and Aqueous by maceration technique. 50 g of *T. diversifolia* powder were macerated in 600 mL of each solvent in 900 ml glass jar and stirred on the laboratory auto VWR orbital shaker® model 1000 for 24 hours at 250 revolutions per minute. After shaking for 24 hours, the mixture of each solvent was then allowed to settle for another 24 hours and then filtered into another glass jar using Whatman 185 µm filter paper. This was to harvest all the active soluble chemicals present with the help of the four solvents (Ethanol, Methanol, N-Hexane and Distilled water) with different polarities. The filtrates gotten with Ethanol, Methanol and n-Hexane were then separately concentrated in a RV 8® rotary evaporator at 65°C at 120 r/min whereas aqueous extract (Distilled water) was lyophilized in a water bath until used. After complete evaporation of the solvents, the different pure fractions of the extracts were stored in air-tight containers and kept in a refrigerator at 5°C till needed for bioassays. The different concentrations of the extracts were prepared by diluting the condensed extracts with acetone, the stock solutions of plant extracts were prepared. Five different concentrations, namely, 2.5; 5.0, 7.5, 10.0 and 15.0% of each extract of *T. diversifolia* were prepared by dissolving the stock solution in the acetone. The 2.5%, 5%, 7.5%, 10.0% and 15.0% concentration of each plant extracts was prepared by diluting 25ml of the extract into 975ml of acetone; 50ml of the extract into 950ml of acetone; 75ml of the extract into 925ml of acetone; 100ml of the extract into 900ml of acetone and 150ml of the extract into 850ml of acetone respect-tively. The prepared concentrations of the extracts were used in the bioassay.

Evaluation of Phytochemical Constituents of Plant Materials

Qualitative phytochemical screening was carried out to ascertain the presence of phytochemicals like phenols, tannins, saponins, triterpenes, flavonoids, alkaloids and steroids in the extracts of *T. diversifolia*. The activity was conducted following the modified procedures of Ajiboye *et al* (2015).

Contact Toxicity Assay by Topical Application

Bioassays were conducted to determine the toxicity of the extracts by topical application. Aliquot of 1 µL of each concentration of extracts was applied topically on the dorsal surface of the thorax of each insect with the aid of micropipette, while the control received 1 µl of acetone. In order to carry out this bioassay, *S. zeamais* and *C. maculatus* were put inside petri dishes and inactivated by keeping them briefly in the refrigerator for three minutes for easy conduct of topical application of the extracts. Each group of ten treated insect individuals was transferred into 10 cm Petri dishes. This experiment was replicated four times and arranged in a Completely Randomized Design (CRD). Insect mortality was recorded at 1, 2, 3, and 7 days after treatment. Abbott's formula (Abbott, 1925) was used to correct for mortality. Corrected mortality values were computed as:

$$Pt = [(Po - Pc) / (100 - Pc)] \times 100$$

Where, Pt = corrected mortality
Po = observed mortality
Pc = control mortality

Repellent Activity Bioassay

The repellency was tested according to modified method of Hamouda *et al*. (2014). Half filter paper discs (Whatman number 40, 9 cm diam.) were prepared and a volume of 100 µl of each plant extract concentration was applied separately to one-half of the filter paper as uniformly as possible with a micropipette. The other half (control) was treated with 100 µl of different extracts. Thereafter, both the treated and the control halves were air dried at room temperature in the laboratory for 10 minutes. Each treated half disc was fitted back to a control half disc with adhesive tape and placed in a petri dish. Twenty adult insects were released in the middle of each filter paper circle. Each concentration was replicated four times. Insects that settled on each half of the filter paper disc were counted after 1 and 2 hours. The average of the counts was converted to percentage repellency (PR) using the formula of McDonald *et al*. (1970) as follows:

$$PR = [(Nc - Nt) / (Nc + Nt)] \times 100$$

Where Nt = number of insects present on the treated half disc
Nc = Number of insects present on the untreated half disc

Toxicity of extracts and F1 Progeny Deterrence

In order to evaluate the mortality and F1 emergence effects of extracts on *S. zeamais* and *C. maculatus* adults, five different application rates (0.05; 0.1; 0.2; 0.4; 0.8 ml for *S. zeamais* and 0.05; 0.1; 0.2; 0.3; 0.5 ml for *C. maculatus*) of the extracts were used. The extracts were mixed thoroughly with 10g maize to ensure uniformity before the introduction of insects. Five pairs of the two insect's species (*Sitophilus zeamais* and *Callosobruchus maculatus*) were introduced into each treatment, and control were replicated three times and arranged in Completely Randomized Design (CRD). Mortality of the insects was recorded 1 day, 2 days, 3 days, 7 days and 14 days after treatment application. All insects were removed after 14 days post treatment and left undisturbed until F1 emergence, starting the sixth week post treatment, were counted and recorded. The reproduction inhibition rate was calculated following Tapondjou *et al.* (2002) as:

Reproduction inhibition rate = Ca-Ta/Ca x 100
Where; Ca= number of newly emerged adult insects in the untreated control
Ta= number of newly emerged adult insects in the treated grains.

Damage Assessment

In order to assess the level of damage *S. zeamais* and *C. maculatus* inflicted on maize grains and cowpea treated with the extracts of *T. diversifolia*, two parameters were used, these include percent grain damage and seed weight loss. Application rates of 0.05; 0.1; 0.2; 0.4; 0.8 ml on 10g of maize; 0.05; 0.1; 0.2; 0.3; 0.5 ml on 10g of cowpea was used for the evaluation for each extract. The extracts were mixed thoroughly with 10g maize and cowpea to ensure uniformity before the introduction of insects. Each treatment and control were replicated three times and arranged in Completely Randomized Design (CRD). Five pairs of adults of *S. zeamais* and *C. maculatus* were thereafter introduced into each treatment and monitored for ten weeks in the laboratory. Damage assessment was measured using percentage grain damage and grain weight loss. The percentage damage and weight loss of grains were measured after ten weeks by counting the damaged and undamaged seeds and was calculated using the formula of Compton and Sherington, (1998):

% damage = Nd/Nu + Nd × 100
Where Nd= Number of damaged seeds
Nu= Number of undamaged seeds
% weight loss = PW1 – PW2/PW1 × 100
Where, PW1 = initial weight of sample before infestation
PW2 = final weight of sample after infestation

Data Analysis

Data on percentages of cumulative mortality, oviposition, repellency, seed damage and weight loss were arcsine-transformed (sin-1√(x/100)) and the number of *F*1 progeny recorded was log-transformed (Log(x+100)). Where x = dosages in %. The transformed data were subjected to the ANOVA using DSAASTAT. The means when significant were separated using Tukey Honestly Significant Difference (HSD) test at P≤0.05. All the values obtained during observation were represented as mean ± SE (Standard Errors).

RESULTS

Evaluation of Phytochemical Constituents of Plant Materials

The phytochemical screening of each fractional extract (N-hexane, methanol, ethanol and aqueous extract) of *T. diversifolia* (whole plant) revealed the presence of phenolic compounds (tannins, flavonoids and total phenols), while glucosides were not detected in N-hexane extract.

Following the procedure of Ajiboye *et al.* (2015), the fractional extract was subjected to qualitative phytochemical analysis to test for the presence of secondary metabolites and phytochemical constituents determined were alkaloids, saponins, glycosides, flavonoids, tannins, terpenoids and phenols (Table 1). The qualitative phytochemical screening test showed that the plant (*T. diversifolia*) contained several active compounds which may propel this plant as a potential insecticide against maize and beans weevil. Each active compound tested may vary in quantity but the whole plant was used and only subjected to qualitative test.

Contact Toxicity Assay by Topical Application

Toxicity of the various extracts applied topically to *S. zeamais* and *C. maculatus* was as summarized in Table 2. There was a significant ($p < 0.01$) difference amongst the treatments and there was also an increase in cumulative toxicity level with an increase in the concentration of the *Tithonia* extracts between the second and the seventh day of the bioassay. The highest insect mortality (86.67 % and 90 %) was observed on the third and seventh days, at 10% and 15% concentration level of the botanicals.

Repellent Activity Bioassay

Insect repellency was detectable 1 hour and 2 hours after exposure and the results indicated that *S. zeamais* and *C. maculatus* differ in their susceptibility to *T. diversifolia* extracts and concentrations under laboratory conditions. Generally, all extracts types repelled the weevils, all the percentage repellency were greater than 50% (Table 3). The different doses of the different fractions of the extracts also showed appreciable repellent abilities. Repellency increased with increasing dose of extract administered. Interestingly, for *S. zeamais* N-hexane at highest concentration level of 15% exhibited highest repellency at 1 h (80.00 ± 4.08) and 2 h (85.00 ± 5.00) while methanol extract at highest concentration level of 15% exhibited highest repellency for *C. maculatus* at 1 h (82.50 ± 6.29) and 2 h (80.00 ± 4.08).

Toxicity of extracts

Significant mortality ($p < 0.01$) was observed for both *S. zeamais* and *C. maculatus* when different dosages of the extracts were applied against them (Tables 4 and 5). N-hexane extract induced highest mortality of 76.67% in *S. zeamais* at day 14. Methanolic extract proved to be the most efficient with a mortality of 90.00 % on *C. maculatus* at day 14 post-treatment. All extracts at higher dosages caused highly significant mortality ($p < 0.01$) of *S. zeamais* and *C. maculatus* when compared with the control.

Oviposition and F1 Progeny Deterrence

The different extracts significantly ($p < 0.01$) reduced the progeny of *S. zeamais* and *C. maculatus* while N-Hexane extract highly inhibited the development of *S. zeamais* with 92 % inhibition rate (Table 6) and Methanol inhibited the development of *C. maculatus* with 76 % inhibition (Table 7). However, all extracts at highest dosage levels inhibited the F1 development of both insect species.

Damage Assessment

The extract types significantly ($P \leq 0.05$) influenced the seed damage and weight loss of maize grains. For *S. zeamais*, the least weight loss and grain damage was recorded in N-hexane extract with a mean value of 0.93% and 8.83%, respectively (Table 8), while the least weight loss and seed damage was recorded in Methanol extract with a mean value of 2.04% and 10.06% respectively for *C. maculatus* at the highest dosage level (Table 9). The control has highest values of seed damage and weight loss.

Table 1: Result of the phytochemical screening of *T. diversifolia* extract (Methanol, Ethanol, N-Hexane and Aqueous)

Phytocompounds	**Plant Extracts of *T. diversifolia***			
	Aqueous	**Methanolic**	**Ethanolic**	**N-Hexane**
Tannin	+	+	+	+
Flavonoids	+	+	+	+
Saponin	-	+	+	+
Phenol	+	+	+	+
Terpenoid	+	+	+	+
Alkaloid	-	+	+	+
Glucosides	+	+	+	-

Key: + = Present, **-** = Absent

Table 2: Contact Toxicity (% ± S.E.) by Topical Application of *Tithonia diversifolia*

		% Mortality							
		S. zeamais				*C. maculatus*			
Treatment	**Dosage**	**Day 1**	**Day 2**	**Day 3**	**Day 7**	**Day 1**	**Day 2**	**Day 3**	**Day 7**
Control	1.00	0.33±0.00 ^{a}	0.33±0.00 ^{a}	3.33±2.72^{a}	3.33±2.72^{a}	3.33±2.72^{a}	3.33±2.72^{a}	6.67±2.72 ^{ab}	10.00±0.00 ^{ab}
	2.50	3.33±2.72^{a}	6.67±2.72 ^{ab}	23.33±2.72 ^{b}	43.33±2.72 ^{bc}	10.00±0.00 ^{ab}	23.33±2.72 ^{b}	30.00±0.00 ^{bc}	53.33±2.72 ^{c}
	5.00	3.33±2.72 ^{a}	13.33±2.72 ^{ab}	23.33±2.72 ^{b}	43.33±2.72 ^{bc}	16.67±2.72 ^{b}	30.00±0.00 ^{bc}	33.33±2.72 ^{bc}	56.67±2.72 ^{c}
Aqueous	7.50	6.67±2.72 ^{ab}	10.00±0.00 ^{ab}	26.66±2.72 ^{b}	50.00±0.00 ^{c}	20.00±0.00 ^{b}	26.67±2.72 ^{b}	36.67±2.72 ^{bc}	60.00±0.00 ^{c}
	10.00	10.00±0.00 ^{ab}	16.67±2.72 ^{b}	26.67±2.72 ^{b}	53.33±2.72 ^{c}	20.00±0.00 ^{b}	30.00±0.00 ^{bc}	33.33±2.72 ^{bc}	63.33±2.72 ^{cd}
	15.00	10.00±0.00 ^{ab}	20.00±0.00 ^{b}	26.67±2.72 ^{b}	56.67±2.72 ^{c}	23.33±2.72	33.33±2.72 ^{bc}	50.00±0.00 ^{c}	66.67±2.72 ^{cd}
	2.50	3.33±2.72 ^{a}	10.00±0.00 ^{ab}	23.33±2.72 ^{b}	43.33±2.72 ^{bc}	13.33±2.72 ^{ab}	23.33±2.72 ^{b}	30.00±0.00 ^{bc}	53.33±2.72 ^{c}
	5.00	3.33±2.72 ^{a}	10.00±0.00 ^{ab}	23.33±2.72 ^{b}	46.66±2.72 ^{bc}	20.00±0.00 ^{b}	33.33±2.72 ^{bc}	36.67±2.72 ^{bc}	60.00±0.00 ^{c}
Ethanol	7.50	6.67±2.72 ^{ab}	10.00±0.00 ^{ab}	26.67±2.72 ^{b}	50.00±0.00 ^{c}	20.00±0.00 ^{b}	26.67±2.72 ^{b}	36.67±2.72 ^{bc}	63.33±2.72 ^{cd}
	10.00	10.00±0.00 ^{ab}	16.67±2.72 ^{b}	26.67±2.72 ^{b}	50.00±0.00 ^{c}	20.00±0.00 ^{b}	30.00±0.00	36.67±2.72 ^{bc}	66.67±2.72 ^{cd}
	15.00	13.33±2.72	20.00±0.00 ^{b}	30.00±0.00 ^{bc}	56.67±2.72 ^{c}	23.33±2.72 ^{b}	33.33±2.72 ^{bc}	53.33±2.72 ^{c}	70.00±4.72 ^{cd}
	2.50	10.00±0.00 ^{ab}	13.33±2.72 ^{ab}	26.67±2.72 ^{b}	43.33±2.72 ^{bc}	16.67±2.72 ^{b}	23.33±2.72 ^{b}	36.67±2.72 ^{bc}	56.67±2.72 ^{c}
	5.00	6.67±2.72 ^{ab}	13.33±2.72 ^{ab}	26.67±2.72 ^{b}	53.33±2.72 ^{c}	20.00±0.00 ^{b}	40.00±0.00 ^{bc}	36.67±2.72 ^{bc}	66.67±2.72 ^{cd}
Methanol	7.50	6.67±2.72 ^{ab}	13.33±2.72 ^{ab}	26.67±2.72 ^{b}	56.67±2.72 ^{c}	23.33±2.72 ^{b}	26.67±2.72 ^{b}	36.67±2.72 ^{bc}	76.67±4.72 ^{cd}
	10.00	10.00±0.00 ^{ab}	20.00±0.00 ^{b}	33.33±2.72 ^{bc}	70.00±4.72 ^{cd}	20.00±0.00 ^{b}	36.67±2.72 ^{bc}	50.00±0.00 ^{c}	86.67±6.52 ^{d}
	15.00	16.67±2.72^{b}	23.33±2.72 ^{b}	40.00±0.00 ^{bc}	83.33±4.72 ^{d}	23.33±2.72 ^{b}	36.67±2.72 ^{bc}	60.00±0.00 ^{c}	90.00±7.72 ^{d}
	2.50	10.00±0.00 ^{ab}	13.33±2.72 ^{ab}	26.67±2.72 ^{b}	43.33±2.72 ^{bc}	16.67±2.72 ^{b}	23.33±2.72 ^{b}	36.67±2.72 ^{bc}	53.33±2.72 ^{c}
	5.00	6.67±2.72 ^{ab}	13.33±2.72 ^{ab}	26.67±2.72 ^{b}	56.67±2.72 ^{c}	20.00±0.00 ^{b}	40.00±0.00 ^{bc}	43.33±2.72 ^{bc}	63.33±2.72 ^{cd}
N-Hexane	7.50	10.00±0.00 ^{ab}	16.67±2.72 ^{b}	30.00±0.00 ^{bc}	66.67±2.72 ^{cd}	23.33±2.72 ^{b}	26.67±2.72 ^{b}	36.67±2.72 ^{bc}	73.33±4.72 ^{cd}
	10.00	10.00±0.00 ^{ab}	23.33±2.72 ^{b}	36.67±2.72 ^{bc}	73.33±4.72 ^{cd}	20.00±0.00 ^{b}	33.33±2.72 ^{bc}	50.00±0.00 ^{c}	80.00±6.52 ^{d}
	15.00	16.67±2.72 ^{b}	26.67±2.72 ^{b}	43.33±2.72 ^{bc}	86.67±6.52 ^{d}	23.33±2.72 ^{b}	36.67±2.72 ^{bc}	56.67±2.72 ^{c}	86.67±6.52 ^{d}

Notes: Each value is the mean ± standard error of four replicates, each replicate with 20 adults. Mean followed by the same letters within the same column are not significantly ($p \leq 0.05$) different from each other using Tukey HSD Test.

Table 3: Percent Mortality (Mean ± SE) of adult *S. zeamais* exposed to varying concentrations of *T. diversifolia*

Mortality					
	Duration of exposure (Days)				
Treatment Dosage(ml)	1	2	3	7	14
Control	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}
Aqueous 0.05	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	3.33±2.72^{a}	10.00±0.00^{ab}
0.1	0.00±0.00^{a}	0.00±0.00^{a}	3.33±2.72^{a}	3.33±2.72^{a}	13.33±2.72^{ab}
0.2	3.33±2.72^{a}	3.33±2.72^{a}	10.00±0.00^{ab}	13.33±2.72^{ab}	23.33±2.72^{bc}
0.4	3.33±2.72^{a}	10.00±0.00^{ab}	10.00±0.00^{ab}	26.67±0.00^{bc}	33.33±2.72^{c}
0.8	10.00±0.00^{ab}	13.33±2.72^{ab}	26.67±2.72^{bc}	33.33±2.72^{c}	43.33±2.72^{cd}
Ethanol 0.05	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	3.33±2.72^{a}	10.00±0.00^{ab}
0.1	0.00±0.00^{a}	0.00±0.00^{a}	3.33±2.72^{a}	6.67±2.72^{ab}	13.33±2.72^{ab}
0.2	3.33±2.72^{a}	3.33±2.72^{a}	10.00±0.00^{ab}	13.33±2.72^{ab}	20.00±0.00^{b}
0.4	3.33±2.72^{a}	6.67±2.72^{ab}	10.00±0.00^{ab}	26.67±0.00^{bc}	36.67±2.72^{c}
0.8	10.00±0.00^{ab}	13.33±2.72^{ab}	26.67±2.72^{bc}	36.67±2.72^{c}	46.67±2.72^{cd}
Methanol 0.05	0.00±0.00^{a}	3.33±2.72^{a}	3.33±2.72^{a}	6.67±2.72^{ab}	10.00±0.00^{ab}
0.1	0.00±0.00^{a}	3.33±2.72^{b}	6.67±2.72^{ab}	10.00±0.00^{ab}	23.33±2.72^{bc}
0.2	3.33±2.72^{a}	6.67±2.72^{bc}	13.33±2.72^{ab}	23.33±2.72^{bc}	43.33±2.72^{cd}
0.4	10.00±0.00^{ab}	13.33±2.72^{ab}	20.00±0.00^{b}	33.33±2.72^{c}	50.00±0.00^{cd}
0.8	16.67±2.72^{b}	26.67±2.72^{bc}	26.67±0.00^{bc}	56.67±2.72^{d}	73.33±2.72^{e}
N-Hexene 0.05	0.00±0.00^{a}	6.67±2.72^{ab}	6.67±2.72^{ab}	6.67±2.72^{ab}	16.67±0.00^{b}
0.1	0.00±0.00^{a}	3.33±2.72^{a}	6.67±2.72^{ab}	10.00±0.00^{ab}	26.67±0.00^{bc}
0.2	3.33±2.72^{a}	10.00±0.00^{ab}	13.33±2.72^{ab}	23.33±2.72^{bc}	46.67±2.72^{cd}
0.4	10.00±0.00^{ab}	13.33±2.72^{ab}	20.00±0.00^{b}	33.33±2.72^{c}	53.33±2.72^{cd}
0.8	16.67±2.72^{b}	26.67±2.72^{bc}	40.00±0.00^{cd}	56.67±2.72^{d}	76.67±4.72^{e}

Notes: Each value is the mean ± standard error of three replicates, each replicate with 10 adults. Mean followed by the same letters within the same column are not significantly ($p \leq 0.05$) different from each other using Tukey HSD Test.

Table 4: Effect of *Tithonia diversifolia* plant extracts on the mortality (% ± S.E.) of adult *Callosobruchus maculatus* in day intervals after treatment

	Mortality				
	Duration of exposure (Days)				
Treatment Dosage(ml)	1	2	3	7	14
Control	0.00±0.00^{a}	3.33±2.72^{a}	3.33±2.72^{a}	6.67±2.72^{ab}	10.00±0.00^{ab}
Aqueous 0.05	6.67±2.72^{ab}	16.67±2.72^{b}	26.67±0.00^{bc}	33.33±2.72^{c}	50.00±0.00^{cd}
0.1	3.33±2.72^{a}	6.67±2.72^{ab}	26.67±0.00^{bc}	46.67±2.72^{cd}	56.67±2.72^{d}
0.2	6.67±2.72^{ab}	16.67±2.72^{b}	33.33±2.72^{c}	50.00±0.00^{cd}	63.33±2.72^{de}
0.3	6.67±2.72^{ab}	10.00±0.00^{ab}	33.33±2.72^{c}	46.67±2.72^{cd}	63.33±2.72^{de}
0.5	10.00±0.00^{ab}	20.00±0.00^{b}	36.67±2.72^{c}	53.33±2.72^{d}	66.67±2.72^{de}
Ethanol 0.05	6.67±2.72^{ab}	16.67±2.72^{b}	33.33±2.72^{c}	40.00±0.00^{cd}	63.33±2.72^{de}
0.1	6.67±2.72^{ab}	6.67±2.72^{ab}	33.33±2.72^{c}	53.33±2.72^{d}	63.33±2.72^{de}
0.2	10.00±0.00^{ab}	23.33±2.72^{bc}	36.67±2.72^{c}	56.67±2.72^{d}	66.67±2.72^{de}
0.3	10.00±0.00^{ab}	13.33±2.72^{ab}	36.67±2.72^{c}	56.67±2.72^{d}	66.67±2.72^{de}
0.5	10.00±0.00^{ab}	20.00±0.00^{b}	36.67±2.72^{c}	56.67±2.72^{d}	70.00±0.00^{e}
Methanol 0.05	10.00±0.00^{ab}	16.67±2.72^{b}	23.33±2.72^{bc}	43.33±2.72^{cd}	66.67±2.72^{de}
0.1	6.67±2.72^{ab}	16.67±2.72^{b}	23.33±2.72^{bc}	46.67±2.72^{cd}	70.00±0.00^{e}
0.2	10.00±0.00^{ab}	16.67±2.72^{b}	40.00±0.00^{cd}	56.67±2.72^{d}	70.00±0.00^{e}
0.3	10.00±0.00^{ab}	26.67±0.00^{bc}	56.67±2.72^{d}	76.67±4.72^{e}	73.33±2.72^{e}
0.5	16.67±2.72^{b}	23.33±2.72^{c}	56.67±2.72^{d}	76.67±4.72^{e}	90.00±0.00^{f}
N-Hexane 0.05	6.67±2.72^{ab}	13.33±2.72^{ab}	23.33±2.72^{bc}	40.00±0.00^{cd}	63.33±2.72^{de}
0.1	6.67±2.72^{ab}	13.33±2.72^{ab}	20.00±0.00^{b}	46.67±2.72^{cd}	63.33±2.72^{de}
0.2	10.00±0.00^{ab}	16.67±2.72^{b}	36.67±2.72^{c}	53.33±2.72^{d}	66.67±2.72^{de}
0.3	10.00±0.00^{ab}	26.67±0.00^{bc}	56.67±2.72^{d}	73.33±2.72^{e}	73.33±2.72^{e}
0.5	13.33±2.72^{ab}	23.33±2.72^{bc}	56.67±2.72^{d}	73.33±2.72^{e}	83.33±2.72^{ef}

Notes: Each value is the mean ± standard error of four replicates, each replicate with 20 adults. Mean followed by the same letters within the same column are not significantly ($p \leq 0.05$) different from each other using Tukey HSD Test.

Table 5: Percentage repellence (Mean ± SE) of *Sitophilus zeamais* due to treatment of maize seeds using different concentrations of extracts of *Tithonia diversifolia*

Treatment	Dosage	% Repellency			
		S. zeamais		*C. maculatus*	
		1 Hour	2 Hour	1 Hour	2 Hour
Aqueous	2.5	57.50±2.50[a]	62.50±4.78[a]	57.50±4.78[a]	55.00±6.46 [a]
	5	60.00±4.08[a]	62.50±2.50[a]	60.00±4.08[a]	62.50±4.78 [a]
	7.5	72.50±2.50[b]	65.00±2.88[a]	60.00±4.08[a]	65.00±6.46 [a]
	10	75.00±2.88[b]	67.50±2.50[ab]	65.00±2.89[a]	72.50±4.78 [b]
	15	77.50±4.78[bc]	70.00±4.78[b]	67.50±4.78[ab]	77.50±4.88 [bc]
Ethanol	2.5	62.50±4.78[a]	67.50±4.78[ab]	70.00±4.08[b]	72.50±4.79 [b]
	5	67.50±4.78[ab]	70.00±4.08[b]	72.50±4.78[b]	75.00±2.89 [b]
	7.5	75.00±2.88[b]	75.00±2.88[b]	77.50±4.78[bc]	75.00±6.46 [b]
	10	72.50±2.50[b]	77.50±4.78[b]	80.00±4.08[c]	80.00±4.08[c]
	15	80.00±4.08[c]	80.00±4.08[c]	82.50±6.29[c]	80.00±4.08[c]
Methanol	2.5	65.00±2.89[a]	67.50±7.27[ab]	65.00±6.46[a]	65.00±2.89[a]
	5	70.00±4.08[b]	72.50±2.50[b]	65.00±6.46[a]	72.50±4.79[b]
	7.5	70.00±4.08[b]	72.50±4.78[b]	70.00±4.08[b]	75.00±6.46[b]
	10	65.00±5.00[a]	72.50±2.50[b]	75.00±6.46[b]	77.50±4.79[bc]
	15	75.00±2.88[b]	72.50±4.78[b]	82.50±6.29[c]	80.00±4.08[c]
N-Hexane	2.5	62.50±4.78[a]	72.50±4.78[b]	62.50±4.79[a]	70.00±4.08[b]
	5	70.00±4.08[b]	75.00±2.88[b]	62.50±4.79[a]	70.00±7.07[b]
	7.5	75.00±2.88[b]	75.00±2.88[b]	70.00±4.08[b]	72.50±4.79[b]
	10	80.00±4.08[c]	80.00±4.08[c]	72.50±4.79[b]	75.00±2.89[b]
	15	80.00±4.08[c]	85.00±5.00[c]	75.00±5.00[b]	77.50±8.54[bc]

Notes: Each value is the mean ± standard error of four replicates, each replicate with 20 adults. Mean followed by the same letters within the same column are not significantly ($p \leq 0.05$) different from each other using Tukey HSD Test.

Table 6: Effects of extracts of *T diversifolia* on emergence (Mean ± SE) of F1 progeny of *S. zeamais*

Treatment Dosage(ml)	F1 Emergence	Reproduction inhibition rate %
Control	52.67 ± 0.94^{a}	–
Aqueous 0.05	39.67 ± 1.97^{ab}	25
0.1	33.67 ± 1.23^{b}	36
0.2	22.57 ± 1.25^{bc}	57
0.4	13.57 ± 0.57^{c}	74
0.8	11.67 ± 0.66^{c}	78
Ethanol 0.05	37.00 ± 1.52^{ab}	30
0.1	28.00 ± 0.98^{b}	47
0.2	19.33 ± 1.25^{bc}	63
0.4	12.66 ± 0.87^{c}	76
0.8	10.00 ± 1.98^{cd}	81
Methanol 0.05	35.33 ± 0.66^{ab}	33
0.1	29.67 ± 1.55^{b}	44
0.2	19.67 ± 1.42^{bc}	63
0.4	13.67 ± 1.79^{c}	74
0.8	7.33 ± 0.57^{cd}	86
N-Hexane 0.05	34.66 ± 0.33^{ab}	34
0.1	27.67 ± 0.57^{b}	47
0.2	12.67 ± 0.47^{ab}	76
0.4	8.67 ± 0.33^{cd}	84
0.8	4.17 ± 0.87^{d}	92

Notes: Each value is the mean ± standard error of four replicates, each replicate with 20 adults. Mean followed by the same letters within the same column are not significantly ($p \leq 0.05$) different from each other using Tukey HSD Test.

Table 7: Effect of *Tithonia diversifolia* on emergence of F1 progeny *Callosobruchus maculatus* of cowpea

Percentage F1 Progeny Emergence			
	Oviposition	F1 Emergence	Reproduction inhibition rate %
Treatment Dosage(ml)			
Control	58.00±0.58[a]	65.00±0.50[a]	–
Aqueous 0.05	46.33±0.34[ab]	53.00±1.00[ab]	18
0.1	38.33±0.34[b]	51.67±1.26[b]	21
0.2	27.00±0.58[bc]	43.33±1.26[bc]	33
0.3	19.33±0.34[bc]	36.67±1.04[c]	44
0.5	13.33±0.34[c]	23.33±0.29[cd]	64
Ethanol 0.05	45.00±0.58[ab]	51.33±0.58[ab]	21
0.1	33.33±0.34[b]	50.67±0.77[b]	22
0.2	25.00±0.00[bc]	43.33±1.25[bc]	33
0.3	12.33±0.34[c]	33.66±1.26[c]	48
0.5	14.67±0.34[c]	21.00±0.58[cd]	68
Methanol 0.05	42.33±0.34[ab]	48.00±0.50[ab]	26
0.1	33.33±0.34[b]	40.00±0.50[b]	38
0.2	23.67±0.34[bc]	36.67±1.04[bc]	44
0.3	10.67±0.34[cd]	23.33±0.77[cd]	64
0.5	2.00±0.34[e]	15.67±0.29[d]	76
N-Hexane 0.05	41.67±0.34[ab]	49.33±0.29[ab]	24
0.1	35.33±0.34[b]	40.00±0.50[b]	38
0.2	25.67±0.34[bc]	36.67±0.77[ab]	44
0.3	16.00±0.58[c]	26.67±0.77[cd]	59
0.5	5.00±0.58[d]	19.33±1.04[cd]	70

Notes: Each value is the mean ± standard error of four replicates, each replicate with 20 adults. Mean followed by the same letters within the same column are not significantly ($p \leq 0.05$) different from each other using Tukey HSD Test.

Table 8: Effects of extracts of *Tithonia diversifolia* on grain damage and weight loss

	Percentage seed damage/weight loss	
	Grain damage	Weight loss
Treatment Dosage(ml)		
Control	42.76±1.46[a]	12.57±1.46[a]
Aqueous 0.05	32.33±0.49[b]	4.33±0.49[b]
0.1	27.76±0.83[b]	4.76±0.83[b]
0.2	22.53±0.46[bc]	3.53±0.46[c]
0.4	18.67±1.33[c]	3.66±1.33[c]
0.8	15.13±0.45[c]	3.02±0.45[c]
Ethanol 0.05	29.33±1.56[b]	4.43±1.56[b]
0.1	25.43±0.43[bc]	3.83±0.43[c]
0.2	20.01±2.09[bc]	3.20±0.09[c]
0.4	15.53±0.53[c]	2.93±0.53[d]
0.8	13.83±2.46[c]	2.43±0.45[d]
Methanol 0.05	25.43±0.43[bc]	4.10±0.43[b]
0.1	23.43±2.91[bc]	3.73±0.19[c]
0.2	18.56±0.81[c]	3.13±0.81[c]
0.4	14.86±2.16[c]	2.10±0.16[d]
0.8	10.06±1.01[d]	1.90±0.01[de]
N-Hexane 0.05	26.63±1.05[b]	4.03±0.05[b]
0.1	23.66±0.81[bc]	3.50±0.81[c]
0.2	18.36±0.36[c]	2.36±0.36[d]
0.4	13.33±0.36[c]	1.33±0.36[de]
0.8	8.83±1.44[d]	0.93±0.44[e]

Notes: Each value is the mean ± standard error of four replicates, each replicate with 20 adults. Mean followed by the same letters within the same column are not significantly ($p \leq 0.05$) different from each other using Tukey HSD Test.

Table 9: Effect of *Tithonia diversifolia* on adult weight loss and seed damage

Percentage seed damage/weight loss		
	Seed damage	Weight loss
Treatment Dosage(ml)		
Control	52.06±1.46[a]	16.67±0.14[a]
Aqueous 0.05	38.07±1.76[b]	6.38±0.01[b]
0.1	31.54±2.09[b]	5.95±0.49[b]
0.2	26.51±1.68[bc]	4.43±0.01[c]
0.3	21.32±1.02[c]	4.19±0.72[c]
0.5	17.43±1.89[c]	3.51±0.17[cd]
Ethanol 0.05	39.48±1.01[b]	6.15±0.57[b]
0.1	29.44±1.25[b]	5.52±0.92[c]
0.2	26.76±1.43[bc]	4.35±0.61[c]
0.3	19.53±1.12[c]	3.86±0.14[cd]
0.5	15.05±2.17[c]	3.78±0.75[cd]
Methanol 0.05	37.51±1.68[b]	6.14±0.83[b]
0.1	30.81±2.10[b]	5.45±0.24[c]
0.2	23.06±2.25[bc]	4.15±0.27[c]
0.3	14.86±0.68[c]	3.09±0.10[cd]
0.5	10.06±1.01[d]	2.04±0.82[e]
N-Hexane 0.05	38.70±0.83[b]	6.49±0.38[b]
0.1	29.32±1.02[bc]	5.21±0.88[c]
0.2	24.29±1.66[bc]	4.52±0.75[c]
0.3	15.24±1.42[c]	3.18±0.40[cd]
0.5	12.81±0.53[c]	2.93±0.09[d]

Notes: Each value is the mean ± standard error of four replicates, each replicate with 20 adults. Mean followed by the same letters within the same column are not significantly ($p \leq 0.05$) different from each other using Tukey HSD Test

DISCUSSION

In this study, the phytochemical screening of plant extract of *T. diversifolia* revealed that among the substances investigated, presence of phenolic compounds was detected (total phenols, tannins and flavonoids), including alkaloids, terpenoids, glycoside and saponins. Thus, the presence of these phytochemicals in plant extract of *T. diversifolia* suggests that the plant might be of insecticidal and medicinal importance. *T. diversifolia* extracts were toxic to both *S. zeamais* and *C. maculatus*. Some secondary plant metabolites may act both as insecticides and antifeedants thereby influencing insect's movement, oviposition, feeding, developmental and physiological processes as well as behavioral patterns as earlier reported by Udo (2011). There was significant ($P < 0.05$) in adult mortality of the beetle in treated seeds with increasing plant extracts. Mortality increased with a longer exposure time as the highest mortality value was recorded in day seven for both topical application and mortality in N-hexane extract and methanolic extract. Therefore, highest mortality at day 7 for *S. zeamais* treated with N-Hexane extract with highest concentration 86.67% and *C. maculatus* treated with Methanol extract with highest concentration 90.00%. This result is similar to the finding of Wachira *et al*. (2014) who observed methanolic leaf extract of Tithonia to be toxic against adult females of *Anopheles gambiae*, and the highest effect was achieved at day 7 of mosquito feeding. N-hexane extract of *T. diversifolia* may act by inhibiting insect acetyl cholinesterase and thus, ultimately blocking the nerve functions. This agrees with studies by Obeng-Ofori and Amitaye (2005) that observed signs of immobilization with flexed legs and clinging to the grain, outstretched meta-thoracic wings from the elytra and paralysis of the dead or dying insects.

There was a significant reduction ($P<0.05$) in emergence of *S. zeamais* and *C. maculatus* in the treated seeds as dosages increased. However, the N-Hexane extract appeared to be more potent than all other extracts in all cases for *S. zeamais* and Methanol extract appeared to be more potent than all other extracts in all cases for *C. maculatus*. The extracts, however, may have been critically toxic to immature stages of this insect given the imbalance between the number of F1 progeny produced and the number of eggs laid (Koona *et al*, 2007). It has also been reported that alkaloids inhibit larval growth and development which by phytochemical screening done in this study shows to be present in both N-hexane extract and Methanolic extract of *T. diversifolia.*

N-hexane extracts and Methanolic extracts of *T. diversifolia* were also able to reduce significantly damage and weight loss in maize and cowpea respectively. Amruta *et al* (2015) recorded effective storage insect control and higher seed quality when treated with botanicals and Emamectin benzoate. This is also in agreement with the findings of Emeasor *et al* (2007) who reported similar work that mixture of seed powder of *Piper guineense* and *Thevetia peruviana* at different percentages caused the highest mortality of *C. maculatus* at 7 days after infestation. There was effect of the extracts on oviposition and F1 emergence as dosages and time of exposure were increased. However, the efficacy of the plant extracts significantly suppressed emergence that can be solely attributed to oviposition properties, which prevent laid eggs from hatching (Mukanga *et al*., 2010). The repellent activity of extracts increased as treatment level increased thereby increasing the protectant potential of *T. diversifolia* against storage insect pest.

CONCLUSION

This study showed the efficacy of botanical extracts as grain protectants against stored products insects. N-Hexane and Methanolic extracts of *T. diversifolia* plants were potent in controlling *S. zeamais* and *C. maculatus* in storage. The protection activity of the stored products in bioassays tested was dose dependent, with higher doses offering better protection. *T. diversifolia* is readily available in the locality of farmers and it pose no threat to humans as it is safe, ecofriendly and can be used for short and long-term storage of the maize grains and cowpea seeds.

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