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RESEARCH ARTICLE

Tiger nut (*Cyperus esculentus*) as a potential phytoinsecticide: larvicidal activity of crude extracts on *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae)

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ABSTRACT

Plants contain bioactive compounds and are constantly explored as safer alternatives to conventional insecticides. Despite numerous studies on many plants, information on the insecticidal potential of underutilised plants like tiger nut, *Cyperus esculentus* L., are scant, although their pharmacological potentials are well known. Hence, this study investigated the larvicidal potential of crude aqueous extracts of two *C. esculentus* varieties (black and yellow) on the mosquitoes *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say). Mosquito larvae were exposed to *C. esculentus* crude extracts using the larval bioassay technique of the World Health Organization. Differential larvicidal responses were observed in the test mosquitoes and extracts of Black Dried Tiger nuts (BDT) were more larvicidal than Yellow Dried Tiger nuts (YDT). Acute larval toxicity of the extracts was more pronounced on *Cx. quinquefasciatus* than *Ae. aegypti*. The results indicate the potential of *C. esculentus* (particularly BDT) as a source of mosquito bioinsecticide and merits further studies as a safer alternative to conventional insecticide-based vector control.

Keywords: *Aedes aegypti*; *Culex quinquefasciatus*; tiger nuts; larvicidal; crude extracts.

INTRODUCTION

Tiger nut, *Cyperus esculentus* L., is regarded to be of tropical and subtropical origin (USDA-ARS, 2014). It is distributed globally and cultivated in several countries but regarded as a weed in others (CABI, 2017). Although a tuber, it shares characteristics of both tubers and nuts (Sanchez-Zapata *et al*., 2012). Tiger nut is very nutritious and used as human food and animal feed in many countries and as raw materials for several industries (Sanchez-Zapata *et al*., 2012). Locally known in Ghana as "atadwe", it is usually consumed raw or in other forms. Milk and edible oil are also extracted from the tubers for various uses (Yeboah *et al*., 2012).

Due to its nutritional composition, tiger nut is associated with several health benefits including anti-inflammatory and analgesic effects as well as the prevention and treatment of diseases (Chukwuma *et al*., 2010; Sanchez-Zapata *et al*., 2012; Adjei-Duodu, 2014). It contains phytochemicals such as alkaloids, sterols, and phenolics (Chukwuma *et al*., 2010; Yeboah *et al*., 2012; Adjei-Duodu, 2014). These compounds are biologically active and efficacious as antimicrobials (Trease & Evans, 2005) and insecticidals (Rey *et al*., 1999; Rahuman *et al*., 2008; Liu *et al*., 2012) based on studies with other plants. Although *C. esculentus* possesses these bioactive phytochemicals, studies on its insecticidal activity are limited (Khater & Shalaby, 2008).

Mosquitoes of the genera *Aedes*, *Anopheles*, *Culex* and *Mansonia* are vectors of several diseases (Foster & Walker, 2019). The yellow fever mosquito, *Aedes aegypti* (L.) and the southern house mosquito, *Culex quinquefasciatus* (Say) are important species of their respective genera. *Aedes aegypti* is responsible for transmitting arboviral diseases like dengue, yellow fever, chikungunya and zika while *Cx. quinquefasciatus* is the vector of many diseases including filariasis. These diseases gravely affect the health of people, accounting for a substantial number of out- and in-patient admissions and deaths. Although the spread of these diseases can be curtailed by several management methods, vector control is the most common.

Implementation of vector control is frequently dominated by the use of conventional insecticides (WHO, 2006; Himidian *et al*., 2012) which has several drawbacks including harmful effects on the environment (Pimentel, 2005). Concerns over conventional insecticides drive research for environmentally-

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sound mosquito biocidals from several sources including plants. Although *C. esculentus* is active against biological organisms especially microbes, it has not been explored as a biological insecticide. This study compared the insecticidal potential of two varieties of tiger nuts on larvae of *Ae. aegypti* and *Cx. quinquefasciatus*.

MATERIALS AND METHODS

Mosquito colonization

Larvae (early 4th instar, susceptible strain) of *Ae. aegypti* and *Cx. quinquefasciatus* were obtained from established colonies at the Vector Control Research Unit of Universiti Sains Malaysia, Penang. The mosquitoes were initially field-collected from Penang, Malaysia over three decades ago and have been maintained insecticide-free under laboratory conditions. The larvae were reared on a diet made up of a mixture (2:1:1:1) of cat biscuit, milk powder, yeast and beef liver as described in El-garj *et al*. (2015) at 27±4°C, 82±7% relative humidity and 12:12 h (L: D) photoperiod in an insecticide-free room with the air-conditioner turned on.

Preparation of phytoextracts

Two tiger nut varieties (black/dark brown and yellowish/light brown) (Figure 1) were purchased from the Madina market in the La-Nkwantanang–Madina municipality in the Greater Accra region of Ghana. They were authenticated (registration number EL001) by the University of Ghana herbarium (Achoribo & Ong, 2019). Each variety was washed with distilled water and sun-dried as whole for 4 days. The tubers, black dried tiger nuts (BDT) and yellow dried tiger nuts (YDT), were milled into powder and stored at -20°C. One hundred grams of each variety was blended with distilled water (500 ml) and filtered with a Daiso® tea bag (Achoribo & Ong, 2019) to obtain the milk (filtrate). The residue was blended with distilled water (500 ml) and filtered. The residue was again blended with 500 ml of distilled water and filtered. The total filtrate (1500 ml) was frozen at -80°C, freeze-dried (Labconco®, USA) and stored at 4°C until ready for larval bioassay.

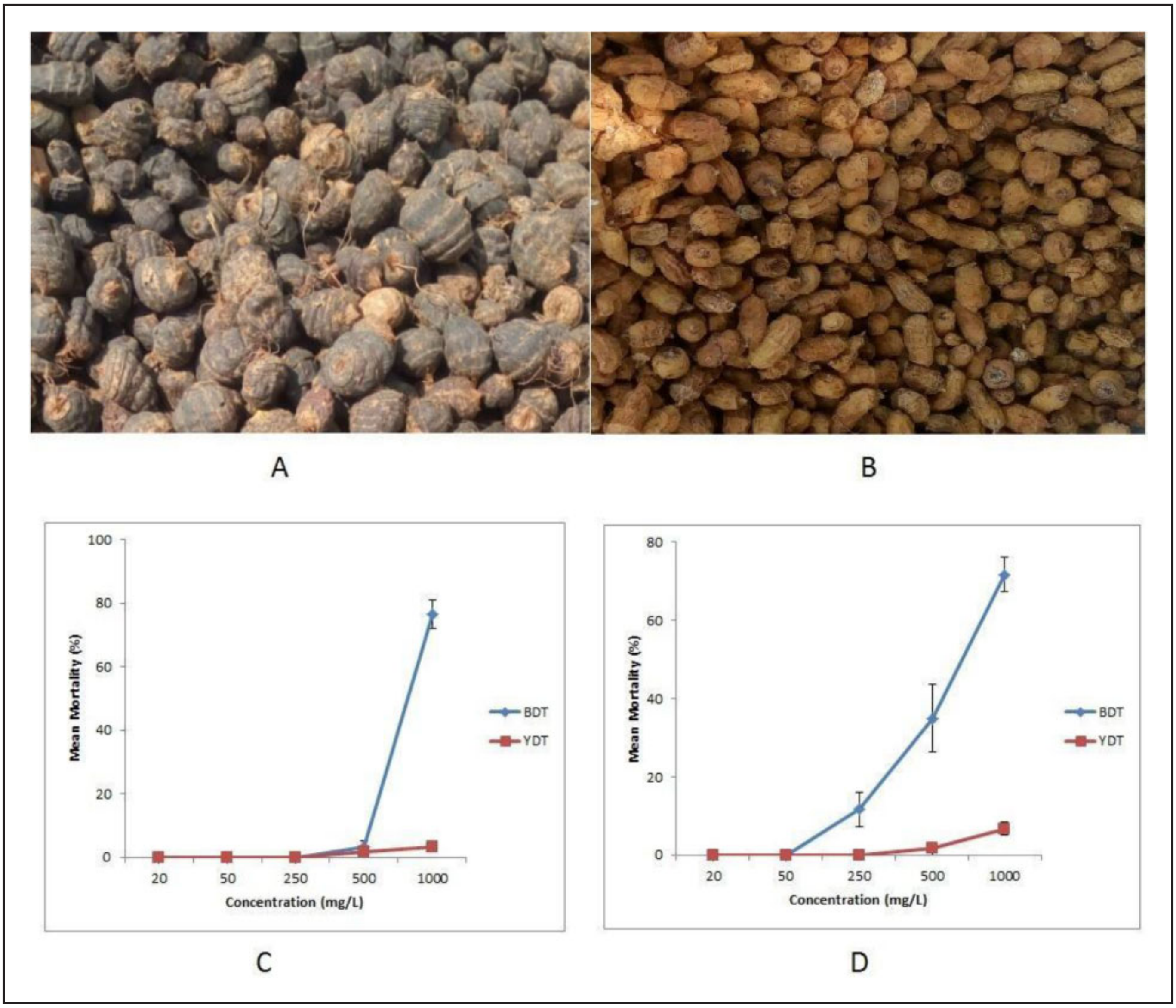

Figure 1. Tiger nut varieties **A**: Black tiger nuts **B**: Yellow tiger nuts.

Larval bioassay

Stock solution (200 000 mg/L) prepared in phosphate buffer saline for each variety was diluted with distilled water to obtain test concentrations of 20, 50, 100, 500, 1 000, 5 000 and 10 000 mg/L. Twenty early 4th instar larvae were exposed (using a dropper) to each concentration in paper cups (top diameter=8.7 cm, base diameter=5.8 cm and height=13.3 cm) in a final volume of 200 ml at a water depth of about 7.2 cm at 26±3°C for 24 h to determine their mortality (WHO, 2005). Larvae that did not display any sign of movement after being probed with a needle were considered dead. The controls were performed using distilled water and phosphate buffer saline (10 mg/L). This was replicated three times.

Data analysis

Arcsine transformation of percentage mortality data was done and subjected to analysis of variance (ANOVA) using SPSS version 20.0. Post hoc test was performed with Tukey HSD if differences were significant at $p<0.05$. General Linear Model (GLM) univariate analysis was used to observe the effect of concentration, variety and mosquito species and their interactions on mortality. QCal software (Lozano-Fuentes *et al*., 2012) was used to analyse dose-response activity in the test mosquitoes. Differences in lethal concentration (LC) at the 50% (LC_{50}) and 90% (LC_{90}) levels were determined by comparing their respective 95% confidence limits (CL) (Abbassy *et al*., 2009).

RESULTS

Aedes aegypti and *Cx. quinquefasciatus* which were exposed to the controls did not exhibit any mortality response. However, the test mosquitoes which were exposed to the tiger nut extracts depicted differences in their mortality responses (Table 1). There was no appreciable increase (<5%) in mortality of *Ae. aegypti* larvae treated with YDT concentration between 20-1 000 mg/L (Table 1). This was also observed in larvae treated with lower concentrations (20-500 mg/L) of BDT. Mortality of *Ae. aegypti* larvae in response to BDT ($p<0.001$) and YDT ($p<0.001$) was significantly different. Larvae of *Cx. quinquefasciatus* treated with the extracts also displayed mortality differences that were significant for BDT ($p<0.001$) and YDT ($p<0.001$).

GLM univariate analysis indicated that mortality responses to concentration ($p<0.001$), tiger nut variety ($p<0.001$) and mosquito species ($p<0.001$) were significantly different (Table 2). Interactions between factors such as concentration*tiger nut variety ($p<0.001$), concentration*mosquito species ($p=0.001$) as well as between all the three factors ($p<0.001$) were significant. However, interaction between tiger nut variety*mosquito species was not significant ($p=0.719$).

Aedes aegypti exposed to BDT and YDT had LC_{50} of 835.01 and 5381.43 mg/L while *Cx. quinquefasciatus* exposed to BDT and YDT had LC_{50} values of 644.11 and 2 973.00 mg/L (Table 3), thus the LC_{50} of *Cx. quinquefasciatus* exposed to both extracts was lower than that of *Ae. aegypti*. The LC_{90} of *Cx. quinquefasciatus* to BDT was higher (1 633.32 mg/L) than that of *Ae. aegypti* (1 164.38 mg/L) while that to YDT (7 078.31 mg/L) was lower than *Ae. aegypti* (17 725.56 mg/L).

DISCUSSION

Plant extracts are screened for insecticidal activities to broaden the range of biocidal tools for insect pest and vector control, however, these have scarcely been done in *C. esculentus*, with most of the biological studies focused on its beneficial properties in the food and pharmaceutical industries (Sanchez-Zapata *et al*., 2012; Yeboah *et al*., 2012; Adjei-Duodu, 2014). In this study, crude extracts of tiger nuts demonstrated different larvicidal effects on *Ae. aegypti* and *Cx. quinquefasciatus*. Mortality of the test mosquitoes ranged from 0 to 100% after exposure to different concentrations of the extracts and this was significantly different among tiger nut varieties, concentrations and mosquito species. Interaction effects among the factors were significant except tiger nut variety*mosquito species interaction. Thus

Table 1. Post exposure (24 hours) mortality of mosquito larvae to crude extracts of tiger nuts

Variety	Concentration (mg/L)	*Aedes aegypti*	*Culex quinquefaciatus*
		% Mortality ± SE	
BDT	20	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	50	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	250	0.00 ± 0.00^{a}	11.67 ± 4.41^{b}
	500	3.33 ± 1.67^{a}	35.00 ± 8.66^{c}
	1 000	76.67 ± 4.41^{b}	71.67 ± 4.41^{d}
	5 000	100.00 ± 0.00^{c}	100.00 ± 0.00^{e}
	10 000	100.00 ± 0.00^{c}	100.00 ± 0.00^{e}
YDT	20	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	50	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	250	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	500	1.67 ± 1.67^{a}	1.67 ± 1.67^{ab}
	1 000	3.33 ± 1.67^{a}	6.67 ± 1.67^{b}
	5 000	50.00 ± 2.89^{b}	75.00 ± 5.77^{c}
	10 000	73.33 ± 3.33^{c}	98.33 ± 1.67^{d}

Mean mortality values with different superscripts in the same column for each variety were significantly different at $p<0.05$ using Tukey HSD. BDT: Black dried tiger nuts; YDT: Yellow dried tiger nuts; SE: Standard error.

Table 2. General Linear Model Univariate analysis of the effect of several factors and their interaction on mortality

Factor	df	MS	F	Sig.
Concentration	6	14139.951	730.298	0.000
Variety	1	7308.936	377.491	0.000
Mosquito species	1	895.785	46.265	0.000
Concentration*Variety	4	993.672	51.321	0.000
Concentration*Mosquito species	4	109.977	5.680	0.001
Variety*Mosquito species	1	2.527	0.131	0.719
Concentration*Variety*Mosquito species	4	269.138	13.900	0.000

df: degree of freedom; MS: Mean Square; F: F value; Sig.: Significance value.

Table 3. Acute toxicity of *Cyperus esculentus* crude extracts on larvae of *Aedes aegypti* and *Culex quinquefasciatus*

Mosquito	Extract	LC_{50} (mg/L)	95% CL	LC_{90} (mg/L)	95% CL	Slope ± SE
Aedes aegypti	BDT	835.01^a	803.20-868.09	1164.38^a	1103.89-1228.19	6.61±0.50
	YDT	5381.43^b	4929.04-5875.23	17725.56^b	15047.99-20879.02	1.84±0.11
Culex quinquefasciatus	BDT	644.11^c	602.39-688.70	1633.32^c	1434.83-1859.38	2.36±0.13
	YDT	2973.00^d	2725.75-3242.50	7078.31^d	6357.56-7880.92	2.53±0.13

Lethal Concentration (LC) values with the same superscript in the same column are not significantly different. CL: Confidence Limits; SE: Standard Error.

concentration*mosquito species and concentration*tiger nut variety interactions significantly influenced mortality. This is similar to Zuharah *et al*. (2014) who observed a significant concentration*plant variety interaction effect on mortality of *Ae. aegypti*. Interaction among all the factors (tiger nut variety*concentration*mosquito species) was also significant in this study. Thus depending on the type of tiger nut extract, the concentration and the mosquito species, the mortality response observed could be different. The activity of BDT on *Ae. aegypti* (LC_{50}=835.01 mg/L) and *Cx. quinquefasciatus* larvae (LC_{50}=654.72 mg/L) is comparable to that of extracts from plants such as *Mangifera indica* (LC_{50}=630.39 mg/L, 95% CL= 523.92-769.15), *Gluta renghas* (LC_{50}=2 854.07 mg/L, 95% CL=2 752.46–2 940.52) and *Melanochyla fasciculiflora* (LC_{50}=2 337.89 mg/L, 95% CL=1 957.51–2 983.31) on *Ae. aegypti* (Zuharah *et al*., 2014). According to Zuharah *et al*. (2014), the performance of *M. indica* highlights its potential as a larvicide compared to the other botanicals. Hence, the activity of BDT in this study indicates its potential as a source of phytoinsecticidal compounds. The larvicidal response by BDT in *Ae. aegypti*, although lower than in *Cx. quinquefasciatus*, was higher than the response evoked by YDT in both mosquito species. Work by Prathibha *et al*. (2014) on mosquito larvae including *Ae. aegypti* and *Cx. quinquefasciatus* using petroleum ether extracts of leaves of *Eugenia jambolana* and *Solidago canadensis* and ethyl acetate extracts of flowers of *Euodia ridleyi* and *Spilanthus mauritiana* evoked stronger larvicidal activities than those recorded in this present study. LC_{50} of the extracts on *Ae. aegypti* ranged from 14.10 to 149.4 mg/L and on *Cx. quinquefasciatus*, it was from 5.103 to 75.31 mg/L. The LC_{90} of the extracts on *Ae. aegypti* and *Cx. quinquefasciatus* also ranged from 33.08 to 195.6 mg/L and 11.0 to 127.4 mg/L. These LC_{50} and LC_{90} values indicate a high efficacy of the above extracts compared to the tiger nut extracts. The BDT extract in this study however had a better efficacy compared to extracts of *Azolla pinnata* (LC_{50}=2 572.45 mg/L) (Ravi *et al*., 2020) and *Clitoria ternatea* (LC_{50}=1 056 mg/L) (Ravindran *et al*., 2020) on early 4th instar larvae of *Ae. aegypti*. Arjunan *et al*. (2012) also observed that ethanol extract of *Aloe vera* leaf induced an LC_{50} of 5 500 mg/L on *Ae. aegypti* and 5 100 mg/L on *Cx. quinquefasciatus*. It also induced an LC_{90} of 12 400 mg/L on *Ae. aegypti* and 11 800 mg/L on *Cx. quinquefasciatus*. With the exception of the LC_{90} of YDT on *Ae. aegypti*, all the LC_{50} and LC_{90} values of the two tiger nut varieties in this study were lower and hence more efficacious than those recorded in Arjunan *et al*. (2012). While assaying for plant extracts with potential insecticidal activity against 3rd instar larvae of *Cx. quinquefasciatus* using distilled water as solvent (Rawani *et al*., 2014), the efficacies of leaves of *Alternanthera sessilis* (LC_{50}=10 300 mg/L), *Gardenia carinata* (LC_{50}=40 700 mg/L), *Ruellia tuberosa* (LC_{50}=45 200 mg/L) and *Trema orientalis* (LC_{50}=17 900 mg/L) were very low compared to the BDT and YDT extracts in this study. Comparisons with the activities of the above phytoextracts draws attention to the potential importance of the tiger nut extracts as bioinsecticides.

Results of the bioassay showed that BDT was more toxic to the test mosquitoes than YDT. Comparing the LC_{50} of the extracts showed that BDT was over 6- and 4-fold more toxic than YDT in *Ae. aegypti* and *Cx. quinquefasciatus* respectively. The mortality differences among the tiger nut varieties may be due to differences in the presence and composition of their phytochemicals. Achoribo and Ong (2019) observed that aqueous extract of BDT had more total phenolic and flavonoid content compared to YDT. In addition to flavonoids and phenolics, both extracts also contained sterols but saponins and terpenoids were only present in BDT and YDT respectively. According to Chukwuma *et al*. (2010), *C. esculentus* contains phytochemicals and these compounds are known from other studies which are not directly *C. esculentus*-related to induce toxicological and other adverse responses in insects (Rey *et al*., 1999; Barbehenn & Constabel, 2011; Liu *et al*., 2012; Ge *et al*., 2015). Larvicidal activity of the extracts may be due to either the action of an individual phytochemical or multiple phytochemicals acting either singly or jointly (Abbassy *et al*., 2009; Ma *et al*., 2014; Ge *et al*., 2015).

Toxicity of the crude aqueous extracts in this study were low compared to those of some essential oils reviewed by Pavela (2015), nonetheless, they compared favourably with other plant extracts as indicated earlier (Arjunan *et al.,* 2012; Rawani *et al*., 2014; Zuharah *et al*., 2014; Ravi *et al*., 2020; Ravindran *et al*., 2020). In Khater and Shalaby (2008), essential oil of *C. esculentus* was more efficacious on 4th instar larvae of *Culex pipiens* from Egypt compared to the effect of the extracts in this study. This could be due to several factors including the solvent, plant part and the mosquito species tested. Bioactives extracted from plant sources could vary depending on the solvent used and the method of extraction (Mojzer *et al*., 2016), so although YDT crude extract induced lower larvicidal responses in the test mosquitoes, these responses could have been different when using other solvents and extraction methods. For instance, the ethanol extract of YDT has a significantly lower total phenolic and flavonoid content compared to its aqueous extract while ethanolic BDT extract has a significantly lower total flavonoid content (Achoribo & Ong, 2019). Tannins were also present in the ethanol but not the aqueous extracts of the tiger nuts. Hence there could be a variation in the toxic responses observed in the test mosquitoes if the bioactives were extracted with different solvents. However, water-based extraction such as in this study has several advantages including the solvent being safe, cheap, abundant and having a wider application (Cater *et al*., 1974; González & Muñoz, 2017).

Culex quinquefasciatus was more susceptible to the extracts compared to *Ae. aegypti*, concurring with studies by Prathibha *et al*. (2014) and Govindarajan *et al*. (2016) that efficacy of phytomosquitocides varies significantly among mosquito species. Using methanol and acetone extracts of *Ocimum canum*, *Ocimum sanctum* and *Rhinacanthus nasutus*, Kamaraj *et al*. (2008) observed higher toxicity in *Cx. quinquefasciatus* compared to *Ae. aegypti*. *Aedes aegypti* and *Cx. quinquefasciatus* are targeted with substantial quantities of conventional insecticides (van den Berg *et al*., 2012), so mortality by the tiger nut extracts (especially BDT) indicates their potential as sources of larvicidal products. Since plant-based insecticides are a valued alternative in the face of increasing conventional insecticide resistance (Vontas *et al*., 2012; Avicor *et al*., 2017) and concomitant toxic effects on the environment and non-target organisms (Pimentel, 2005), larvicidal performance of the tiger nut extracts offers a promising alternative. Studies of its effects on non-target organisms will also be important in assessing its selectivity for pest control.

CONCLUSION

Crude extracts of *C. esculentus* tubers induced mortality in larvae of *Ae. aegypti* and *Cx. quinquefasciatus*. The larvicidal activity of the extracts indicates that *C. esculentus* is a potential source of insecticidal compounds for vector control and presents a safer alternative method of chemical control compared to conventional insecticides. This study underscores the need for further studies on the utility of this underutilised plant as a biolarvicide.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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