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Studies on the Insecticidal Activities of *Pseudocedrela kotschyi* on *Culex quinquefasciatus* Larvae and the Maize Weevil (*Sitophilus zeamais*) Motschulsky

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Abstract

Culex quinquefasciatus is the vector that transmits the causative agent of filariasis which poses a great threat to the world at large. Storage pest are nuisance to farmers because they destroy farm products either in the store or in the farm. In this study, the insecticidal activity of *Pseudocedrela kotschyi* stem bark extract was tested against the fourth instar larvae of *Culex quinquefasciatus* and adult *Sitophilus zeamais.* A total of three hundred and sixty 4th instar larvae of *Culex quinquefasciatus* were divided into six groups each consisting of three replicates containing twenty larvae each. Groups one to four were each administered one of the following dose: 1000mg, 250mg, 125mg and 62.5mg of the ethanolic stem bark extract of *P. kotschyi.* Group five was treated with a standard drug (permethrin 0.6%) and the last group was neither treated with the extract nor the standard drug. The highest concentration 1000mg of the plant extract had 98.33% mortality followed by 250mg which exhibited 61.67%, 125mg with 48.33% mortality and 62.5 with 25%. There was 100% mortality in the group treated with standard drug and no mortality in the control. Analysis of variance showed significant difference ($p<0.05$) in the mortality of larvae between the different extract levels.The mortality of the *Sitophilus zeamais* was observed for 7 daysafter exposure. The highest percentage mortality of 53.33% was recorded inthe weevils treated with 750mgof the plant extract followed by 1750mg which had 50% and the lowest concentration of 500mg plant extract had the lowest mortality of33.33%. However, there is no significant difference ($p>0.05$) between the mortality of the groups administered extract at different concentrations. From the result, it can be tentatively concluded here; based on this research that *P. kotschyi* can act as a bio-pesticide of plant origin which will be less toxic to man and other organisms.

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Introduction

Mosquito-transmitted diseases remain a major cause of the loss of human life worldwide with more than 700 million people suffering from the diseases annually (Taubes, 1997). *C. quinquefasciatus* (Diptera: Culicidae) is widelydistributed in tropical and subtropical areas and is the most important vector of filarialparasite *Wuchereria bancrofti*, although *Anopheles gambiae* s.l and *An. funestus* also play arole in selected areas (Maxwell *et al.*, 1990). *Culex quinquefasciatus*, a vector of lymphatic filariasis is widely distributed in tropical zones with around 120 million people infected wordwide and 44 million people having infected worldwide and 44

million people having common chronic manifestation (Bernhard *et al.,* 2003). Despite its debilitating effects, lymphatic filariasis is given a very low control priority (Ramarah *et al.,* 2000; Yadouléton *et al.,* 2015).

Apart from mosquito as a nuisance to humanity, insects like the maize weevil (*Sitophilus zeamais*) and cowpea weevil (*Callosobruchus maculatus*) also cause great nuisance to humanity by destroying stored grains. The maize weevil (*Sitophilus zeamais*), known in the United States as the greater rice weevil is a species of beetle in the family curculionidae. It can be found in numerous tropical areas around the world and in the United States and is a major pest of maize. This species attacks both standing crops and stored cereal products, including wheat, rice, sorghum, oats, barley, rye, buck-wheat, peas and cotton seed. (Pest web, 2010). Corn, *Zea mays* belongs to the family graminae. It is a cereal grass related to wheat, rice, oat and barley, ranking second after wheat and is followed by third-ranking rice in order of world's grain production. This plant is regarded as versatile and with many uses since it can thrive in diverse climates, hence, it is grown in many countries than any other crop. Aside from being one of the major sources of food for both human and animals, it is also processed into various food and industrial products including starches, sweeteners, oil, beverage, industrial alcohol and fuel ethanol. Therefore, the conservation of this grain is essential to have this basic food available on an ongoing bass (Huang and Subramanyam, 2005).

However, post harvest storage of maize is greatly constrained by the pest, *Sitophilus zeamais* motschulsky. Initial infestations of maize grain occur in the field just before harvest and the insects are carried into the store where the population builds up rapidly (Adedire and Lajide, 2003). Infestations not only cause significant economic losses due to the consumptions of grains, they also result in elevated temperature and moisture conditions that lead to an accelerated growth of molds, including toxigenic species (Maga *et al.,* 2003). The growth of molds leads to the production of aflatoxin.

Stored grains may suffer serious attack from pests (insects, rodents and birds) and pathogens (bacteria and fungi), especially when not protected and when storage hygiene is poor. Amidst other constraints of maize production, insect pests constitute a major threat, destroying approximately 20% of food produce (Pimental, 2007). The damage caused by post harvest pests is much higher than that caused by other agents like rodents and micro-organisms.

The protection of stored products against attack by insect pests is essential in many countries, particularly those that do not have adequate storage facilities. Control of these pests relies on the widespread use of various synthetic chemical insecticides and fumigants. It has led to a number of serious problems such as environmental pollution, pesticide residue in food grains, pesticide resistance and toxicity to non-target organisms (Cosimi *et al.,* 2009). Botanical pesticides are an important group of naturally occurring, often slow-acting crop protectants that are usually safer to humans and the environment than conventional pesticides and with minimal residual effects. Moreover, the fact that botanical pesticides contain mixtures of biologically active substances, no resistance is developed in pests and pathogens. Therefore, the use of plant pesticides has been recommended ever more as a suitable alternatives of plant protection with minimum negative risks (Isman, 2006; Pavela, 2007).

Pseudocedrela kotschyi has numerous uses in traditional medicine, particularly its bark, roots and leaves. The bark of *P. Kotschyi* contains a bitter non-nitrogenous principle, pseudocedrelin, demonstrated to possess piscidal activity (Oliver-Bever, 1986). *P. kotschyi* root extracts have been shown to inhibit the In vitro growth and development of the Schizont stage of *Plasmodium falciparum* and may provide affordable means of treating malaria (Kassim *et al.,* 2009).

The ethanol stem bark extract of *P. kotschyi* reduced the activities of both larvae and adult *Dermestes maculatus* beetles in feeding and movement during the 7 days exposure (Yakubu *et al.,* 2015). Yadouléton *et al.,* (2015) showed that wild populations of *Cx. quinquefasciatus* have developed resistance against pyrethroids, organochlorine and carbamate. There has been an increasing search for edible, cheap, and safe plant materials that will not contaminate food products in acting as grain protectants in small storage systems (Arannilewa, 2002). It is on this basis that the present study was carried out to investigate the insecticidal effects of *P. Kotschyi* against mosquito and maize weevil.

Materials and Methods

Study area

The study was conducted in the Undergraduate Laboratory, Department of Zoology, University of Jos, Jos, Nigeria.

Sample collection

The Mosquito Larvae

The larvae of *Culex* species were identified using the principal characters for mosquito identification (*http://entomology.unl.edu/urbanent/mosquito.htm*). Contrary to the *Anopheles* larvae which lie parallel to the surface of the water, *Cx. quinquefasciatus* larvae hang at an angle to the surface of the water. The collection of larvae was carried out as described by Yadouléton *et al.*, (2015). First instar larvae of mosquito larvae was collected from stagnant water from a compound in the University of Jos students' Village Hostel. Zooplanktons and phytoplankton were collected alongside the larvae in large quantity to serve as feed for the larvae and to act as if in their natural environment. Larvae were collected using the dipping on breeding sites and then kept in labeled bottles. In 2-3 days, the 1st instar larvae metamorphosed into the 4th instar larvae.

The maize weevil

The maize weevil was collected from an old infested stored grain. The weevils were identified based on morphological characters described by Barro and Delong (1964); Hill (1975). The identified weevils were kept in a plastic container covered with muslin cloth along with dried maize grains.

Plant and Extraction

This was carried out in a similar method as described by (Dawet *et al.*, 2011). *Pseudocedrela kotschyi* (Plate 1) stem bark was collected from Bwai in Mangu Local Government Area, Plateau State, Nigeria. The stem bark of *P.kotschyi* was collected and dried in the open air under shade for 7 days and pulverized using mortar and pestle. The pulverized stem bark (220g) was soaked in 400 ml absolute ethanol for 72 hours and filtered. The filtrate was then dehydrated in a hot oven at 50 C and the residue was stored at 4 C until use.

Formulation of the test solution

For the mosquito larvae

5g of the powdered extract was dissolved in 10mls of water which became the stock solution to be used in the test. Various milliliters (ml) of the stock were obtained using 0.1 – 1.0ml calibrated syringe. These quantities of the measured extract were then added into 50ml of straw water for each container. The quantities were 2ml, 0.5ml, 0.25ml, 0.125ml, giving four (4) doses of the extract: 1000mg, 250mg, 125mg and 62.5mg per 50ml respectively; a standard drug with 0.6% permethrin and a control.

For the maize weevils

15g of the powdered extract was dissolved into 30mls of water. The reason for dissolving in distilled water is to increase the moisture content of the maize grains for penetration of the extract into the grains. This severed as the stock. Various milliliters (ml) of the stock solution were measured using 5ml calibrated syringe. The concentrations were 3.5ml, 3ml, 2.5ml, 1.5ml, 1ml, a standard pesticide containing 2.0% permethrin and control. These made it five (5) concentrations of the extract of 1750mg, 1500mg, 1250mg, 750mg and 500mg respectively.

Insect bioassay

50mls of water each, containing phytoplankton and zooplanktons was added into 18 plastic disposable containers each (Plate 2). Then 20 fourth instar larvae of mosquitoes each was added into each container. They were divided into 6 groups consisting of 3 replicate each. Groups 1-4 were administered 2ml, 0.5ml, 0.25ml and 0.125ml of *Pseudocedrela kotschyi* plant extract respectively. Group 5 was treated with 2ml of the standard drug containing 0.6% permethrin. Group 6 was neither treated with the plant extract nor the standard drug. The mortality of larvae was recorded for three (3) consecutive days.

In another set, 20g each of uninfected maize grains was measured into 21 disposable plastic containers each (Plate 3). They were divided into 7 groups consisting of 3 replicates each. Groups 1-5 were administered 3.5ml, 3ml, 2.5ml, 1.5ml and 1ml of the *Pseudocedrela kotschyi* plant extract respectively. Group 6 was treated with 2ml of the standard drug containing 2.0% permethrin. Group 7 was treated with neither the plant extract nor the standard drug. The mortality of the adult weevils was recorded for seven (7) consecutive days.

Determination of the mortality

The mortality of test organisms was observed and recorded every twenty four hours. Mosquito larvae and adult weevils were considered dead when no response was observed after probing them on their antennae,

ventral part or the lateral side with a forcep under a hand lens or dissecting microscope. Death larvae and adults were isolated and stored in separate containers as soon as the mortality was observed and recorded.

Statistical analysis

Data were analyzed using one way analysis of variance (ANOVA). $P<0.05$ were considered significant.

Results and Discussion

LD_{50} of *Culex quinquefasciatus* Larvae After 24 hours

The administration of the ethanolic stem bark extract of *Pseudocedrela kotschyi* at 62.5mg/ml did not result to mortality of *C. quinquefasciatus* larvae after 24hrs ofexposure. However the administration of 125mg/ml, 250mg/ml, and 1000mg/ml resulted to 18.33%, 20%, and 66.67% mortality of *C. quinquefasciatus* larvae respectively after 24 hours of exposure (Table 1).

The LD_{50}, that is, the lethal dose that killed 50% of the mosquito larvae population in 24 hours was 630.95mg/ml (figure 1).

The effect of *Pseudocedrela kotschyi* extract on the three days mortality of *Culex quinquefasciatus* larvae was recorded as seen in table 2.

It was observed that the number of mortality increased with increase in the dose of the plant extract. Analysis of variance shows that there was significant difference ($p<0.05$) between the mortality of the groups.

Effect of *P. kotschyi* ethanolic stem bark extract on *Sitophilus zeamais*

The mortality of the *Sitophilus zeamais* after seven days of administration of the ethanolic extract of *Pseudocedrela kotschyi* is shown in Table 4.

The result showed that the mortality of weevils was not dose dependent since the percentage mortalityof weevils exposed to plant extract at 750mg/ml and 1750mg/ml was 53.33% and 50%respectively (Table 5).

There was high mortality (100%) in the group given the standard drug. However, analysis of variance showed that there is no significant difference ($p>0.05$) in the mortality of weevils exposed to *P. kotschyi* stem bark extract compared with the control.

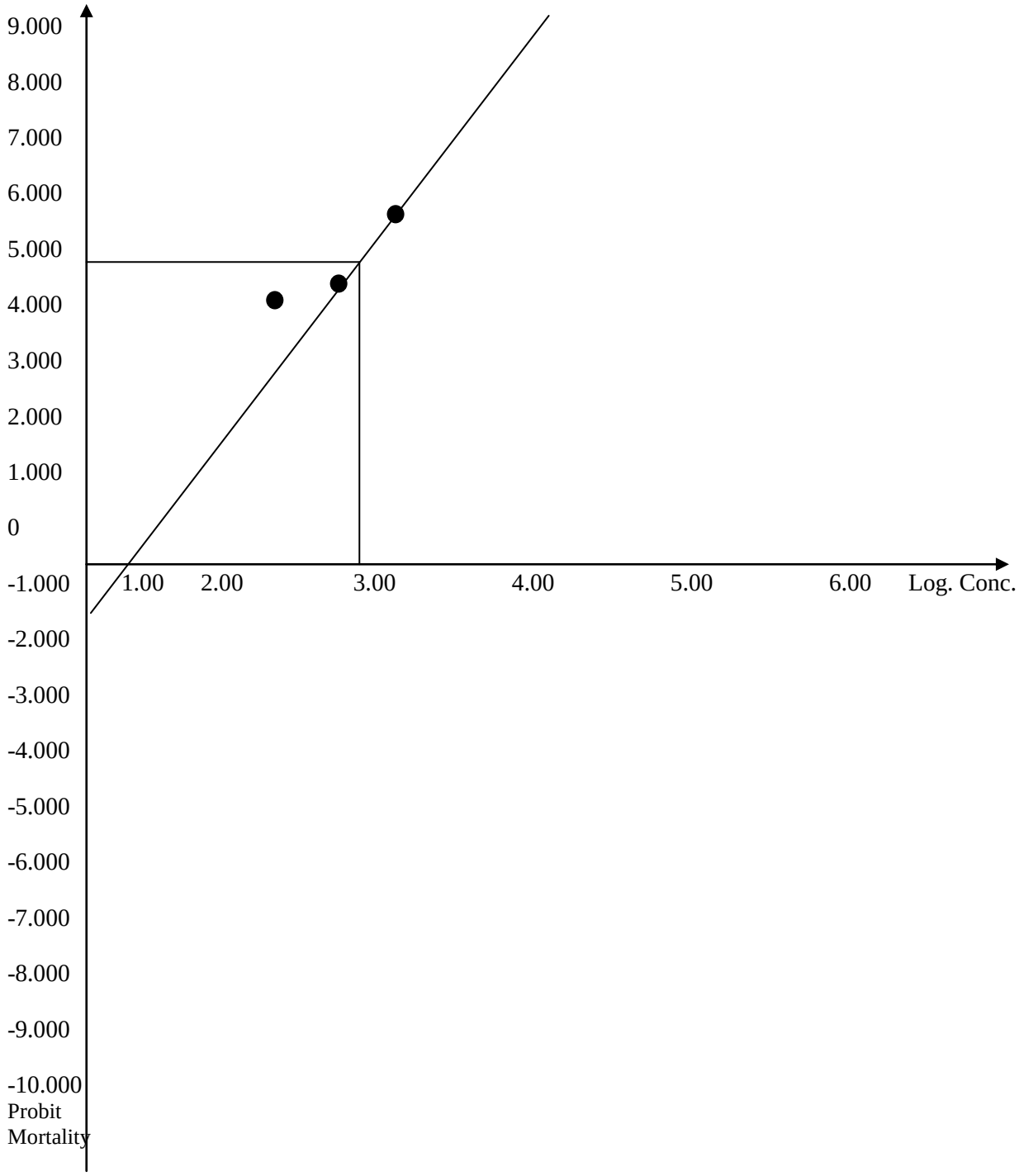
The ethanolic stem bark extract of *Pseudocedrela kotschyi* showed very low to non-effect on *Sitophilus zeamais*, however, the effect on *Culex quinquefasciatus* larvae was very significant. It was observed that during the period of the research, developmental processes like, pupation and metamorphosing to adult was not hindered in *Culex quinquefasciatus* larvae. The high percentage mortality of *Culex quinquefasciatus* recorded in this study agrees with Mankale (2010) who reported 92.30% mortality of *Culex quinquefasciatus* larvae after 24 hours of the administration of 500mg of Neem extract. This results also concord with Gutierrez *et al.,* (2014)whoin their study observed that the three plant *Jatropha curcas*, *Citrus grandis* and *Tinospora rumphii* samples showed larvicidal activity against the dengue-vector, *Aedes aegypti* mosquito larvae which is manifested by a high percentage of mortality in comparison to those in the control group. The mortality of mosquito larvae between the various concentrations of the plant extracts and the control group was also significantly different at 0.05 level of significance. Furthermore, *Tinospora rumphii* leaf extract shows the most effective larvicide among the various plant extracts with the percentage mortality of 90% and 93% in 24 and 48 hours of exposure respectively.

It has been reported that the petroleum ether extract of *Rhinacanthus nasutus* possessed larvicidal effect with LC_{50} values between 3.9 and 11.5mg/L and *Derris elliptica* showed LC_{50} values between 11.2 and 18.84mg/L against *Aedes aegypti, Culex quinquefasciatus, Anopheles dirus and Mansonia uniformis* (Komalamisra *et al.,* 2000). Karmegan *et al.,* (1997) reported that the ethanolic extract of *Jatropha curcas* resulted to 100% acute mortality each at 1000, 500, and 250ppm against *Culex quinquefasciatus* larvae.

The essential oil of aqueous solutions of the stalks and leaves of *Croton argurophylloides, Croton nepetaefolius, Croton sondenanus* and *Croton zehntnen* showed 100% mortality at 50ml against *Aedes aegypti.* (Lima *et al.,* 2006).

The oil from *Cinnamomum camphora, Boswellia carteri, Anethum graveolens* and *Myrtus communis* showed 100% mortality at 50ppm within 3 hours against the 3rd instar larvae of *Aedes aegypti* (Amer and Mehlhorn, 2006). In the present study *Pseudocedrela kotschyi* exhibited a moderate larvicidal activity against *Culex quinquefasciatus* (LD_{50}=630.95mg/ml), and the percentage mortality for the highest concentration after 24 hours was 66.67%.

Fig.1 Probit Mortality/ Log Concentration Graph of *C. quinquefasciatus* Larvae After 24 Hours



Log conc. =2.81; Antilog of 2.81=630.95; LD_{50}= 630.95mg/ml

Plate.1 The Experimental Plant *Pseudocedrela kotschyi*

Plate.2 Experimental Set up Showing *Culex quinquefasciatus* in Plastic Containers

Plate.3 Experimental Set up Showing Maize Infested with *Sitophilus zeamais* in Plastic Containers

Table.1 Probit Mortality of *Culex quinquefasciastus* Larvae After 24 Hours

CONC. Mg/ml	No. of larvae	Mean mortality after 24 hrs	Percentage mortality	Log concentration	Probit mortality
1000	**20**	**13.33**	**66.67**	**3.00**	**5.4316**
250	**20**	**4.00**	**20.00**	**2.39**	**4.1584**
125	**20**	**3.67**	**18.33**	**2.09**	**4.0884**
62.5	**20**	**0.00**	**0.00**	**1.79**	**0.0000**

Table.2 Mortality of *C. quinquefasciatus* Larvae at Different Concentrations of the Ethanolic Extract of *P. kotschyi* After 3 days

Conc. Mg/ml	Replicates	No. of larvae	Mortality			Total mortality
			Day 1	Day 2	Day 3	
1000	A	20	15	5	-	20
	B	20	12	8	-	20
	C	20	13	6	-	19
250	A	20	4	3	5	12
	B	20	5	4	4	13
	C	20	3	2	7	12
125	A	20	0	2	4	6
	B	20	5	2	5	12
	C	20	6	0	5	11
62.5	A	20	0	0	5	5
	B	20	0	0	6	6
	C	20	0	0	4	4
Standard drug	A	20	20	-	-	20
	B	20	20	-	-	20
	C	20	20	-	-	20
Control	A	20	0	0	0	0
	B	20	0	0	0	0
	C	20	0	0	0	0

Table.3 Mean Mortality of *C. quinquefasciatus* Larvae at Different Concentrations of the Ethanolic Extract of *P. kotschyi* After 3 days

Conc. Mg/ml	No. of larvae per replicate (60/3)	Mean mortality ±SE	Percentage (%) mortality
1000	20	19.67±0.33	98.33
250	20	12.33±0.33	61.67
125	20	9.67±1.86	48.33
62.5	20	5±0.58	25.00
Standard drug	20	20±0.00	100.00
Control	20	0±0.00	0.00

Table.4 Mortality of *sitophilus zeamais* at different concentrations of the ethanolic extract of *Pseudocedrela kotschyi* after 7 days

Conc. Mg/ml	Replicates	No. of weevils	Mortality of *sitophilus zeamais*							Total mortality
			Day 1	Day2	Day3	Day4	Day5	Day6	Day7	
1750	A	10	0	0	1	1	0	1	2	5
	B	10	0	0	1	0	1	0	1	3
	C	10	0	3	0	1	0	2	1	7
1500	A	10	0	2	0	1	1	2	3	9
	B	10	1	2	0	0	0	0	0	3
	C	10	0	0	0	0	1	1	0	2
1200	A	10	0	0	0	0	0	1	4	5
	B	10	0	0	1	1	1	2	0	5
	C	10	0	0	1	0	0	0	1	2
750	A	10	0	0	0	0	3	0	0	3
	B	10	0	0	0	1	3	4	1	9
	C	10	0	1	2	1	0	0	0	4
500	A	10	0	0	0	0	0	0	2	2
	B	10	0	2	0	0	0	2	0	4
	C	10	0	1	1	1	0	0	1	4
Standard drug	A	10	10	-	-	-	-	-	-	10
	B	10	10	-	-	-	-	-	-	10
	C	10	10	-	-	-	-	-	-	10
Control	A	10	0	0	0	0	0	0	0	0
	B	10	0	0	1	0	0	1	0	2
	C	10	0	0	0	1	0	1	1	3

Table.5 Mean Mortality of *Sitophilus zeamais*

Conc. Mg/ml	No. of weevils per replicate(30/3)	Mean mortality ±SE	Percentage(%) mortality
1750	10	5±1.15	50.00
1500	10	4.67±2.19	46.67
1200	10	4.00±1.00	40.00
750	10	5.33±1.86	53.33
500	10	3.33±0.67	33.33
Standard drug	10	10±0.00	100.00
Control	10	1.67±0.88	16.67

From this study, it has been observed that *Pseudocedrela kotschyi* demonstrated moderate to high effect on the mosquito larvae and low effect on the maize weevil. This is at variance with Adeniyi *et al.,* (2010) who reported no appreciable effect of *Pseudocedrela kotschyi* on *Streptococcus mutans* and *Staphylococcus aureus.* There were no zones of inhibition at all concentrations tested. The results of this study is not consistent with Azokou *et al.,* (2013) who investigated the larvicidal activities of 45 plants including *P. kotschyi*, traditionally used in Côte d'Ivoire. Of the 49 ethanol crude extracts 7 (14.29%) showed high activity against III and IV instar larvae of *Anopheles gambiae* and *Culex quinquefasciatus* at 1000 ppm 24 h post-exposure. These seven extracts were obtained from six plant species: *Cissus populnea*, *Cochlospermum planchonii*, *Heliotropium indicum*, *Phyllanthus amarus*, *Vitex grandifolia* and *Alchornea cordifolia*. However, three most active plant species (LC50 = 80–180 ppm) were *Cs. populnea*, *Cm. planchonii* and *P. amarus*. Six of the extracts had effect on viability of susceptible and resistant larvae of *Anopheles*, resulting in death of larvae.

The effect of *Pseudocedrela kotschyi* on the mortality of *Sitophilus zeamais* shows that there is no significant difference ($p>0.05$). However, to date, numerous plant extracts are shown to possess strong feeding deterrent (Arnason *et al.,* 1987) and mortality activity. In this study it can be stated that *Pseudocedrela kotschyi* have very low effect on *Sitophilus zeamais* compared with *Culex quinquefasciatus.*

The report of this study is consistent with Yakubu *et al.,* (2015) who reported that the ethanol stem bark extract of *P. kotschyi* reduced the activities of *D. maculatus* larvae and adult in feeding and movement within infested fish samples during the days of exposure, suggesting that the plant possesses potential insecticidal properties although low mortality of *D. maculatus* larvae and adults were recorded at varying concentrations. This study corroborates with Boussaada *et al.,* (2008) who reported that larval growth inhibition of flour beetle *Tribolium confusum* was significantly induced by methanolic and ethyl acetate extracts of *Mantisalca duriaei* and petroleum ether, chloroformic and methanolic extracts of *Rhaponticum acaule.* Larvae appeared to be more sensitive than adults which reached respectively 83%, 77% by using petroleum ether and methanol extracts of *R. acaule.*. These adults gave 33% mortality after sixteen days when *R. acaule* petroleum ether was applied.The extracts of the four plants commonly used in traditional medicine in Morroco disrupted the developmental cycle of *Tribolium castaneum* was reported by Jbilou *et al.,* (2006). Significant insecticidal activity against *T. castaneum* larvae and adults was observed with crude methanol extract from *P. harmala*, followed by extracts of *A. iva*, *Ari. baetica* and *R. raphanistrum*. The larvae were more susceptible than adults to extracts of *Ari. baetica* and *R. raphanistrum*.In the manner, Souza *et al.,* (2010) observed that the extracts assy of *Tapirira guianensis, Schinus terebinthifolius, Tabebuia heptaphylla* and *Gomphrena elegans* against *Sitophylus zeamais* using wheat grains, showed no mortality during the first two days. Significant differences from the fifth day and become more pronounced on the tenth day. *T. guianensis* extracts produced the lowest mortality of 13%, followed by *S. terebinthifolius* with 18% mortality and *T. heptaphylla* which gave 35% mortality. All extracts of *G. elegans* led to mortality rates ranging from 36% to 46.55% only on the tenth day.

However, the results of this study is at variance with Sha-sha *et al.,* (2012) who observed that the essential oils of *Artemesia giraldii* and *Artemesia subdigitata* flowering aerial parts exhibited contact toxicity against *Sitophilus zeamais* adults with LD_{50} values of 40.51µg/adult and 76.34µg/adult, respectively. Othira *et al.,* (2009) reported that at a concentration of 2µL air, *Hyptis* oil caused 68% mortality of *Sitophilus zeamais* in 2 days. The variation could probably be due to concentration of the extracts which at low rate may not be effective or the phytochemical composition of the plant extracts might contained active ingredients which may affect insects according to species and stages or the physiology of the adults weevils which might have develop some resistance due to constant exposure to pesticides.

From the research, it suggests that *Pseudocedrela kotschyi* extract significantly exhibited more insecticidal effect on *Culex quinquefasciatus* larvae than no the *Sitophilus zeamais.* Therefore, the effect of the plant extract on the larvae and their potential local availability make it attractive candidate for biopesticides.

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