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Efficacy of *Xylopia aethiopica* ethanolic and aqueous extracts on the control of *Sitophilus oryzae* in stored rice grain

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Stored product insects reduced the quantity, quality, nutritive value and viability of stored crops such as maize, sorghum, wheat and rice. These pests and many others threaten food security. The study investigated the efficacy of *Xylopia aethiopica* in the control of *Sitophilus oryzae* on stored rice. The extracts of this plant were made using ethanol and aqueous as solvent and prepared at the concentrations of 3, 6, 8 and 0 g. Zero gram is the untreated grain that served as the control. Five pairs of male and female each of a day old adult of *S. oryzae* were introduced into jars containing 20 g of rice each and were observed daily for 6 weeks for mortality, oviposition, developmental stages and natality. The phytochemical analysis of the extracts revealed that alkaloids, glycosides, flavonoid and polyphenol which was moderately high and which exposes the active ingredient of the extracts. The phytochemical analysis of the extracts revealed that alkaloids, glycosides, saponins, flavonoids, reducing compounds and polyphenols were present in the extracts in moderate quantities. The proximate analysis of the grain revealed that the carbohydrate content (83.45 ± 0.1) of *Oryza sativa* followed by moisture (7.33 ± 0.1), the least nutrient of proximate analysis was seen in ash (1.00 ± 0.00). Out of the two extraction methods employed, the ethanol extract was a more effective method and thus recommended.

Key words: *Xylopia aethiopica*, ethanolic and aqueous, *Sitophilus oryzae*, rice grain.

INTRODUCTION

Insect pest damaged stored grains result in major economic losses and in Africa where subsistence grain production supports the livelihood of majority of the population, grain loss caused by storage pest such as the rice weevils *Sitophilus oryzae* (Ulebor et al., 2011) is a serious issue. Stored product insects reduce the quantity,

quality, nutritive value and viability of stored crops like maize, sorghum, wheat and rice (Okonkwo, 1998). These pests and many others, threaten food security (Ulebor et al., 2011). Control of pests in stored grains is a serious problem in developing countries in the tropics due to favorable climatic conditions and poor storage structures

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(Bekele and Hasanali, 1997). *Sitophilus* is a genus of weevils. Some species are familiar as pests of stored food products.

Notable species include the rice weevil (*S. oryzae*), wheat weevil (*Sitophilus granarius*) and maize weevil (*Sitophilus zeamais*) (Shadia, 2011).

The rice and maize weevils have a nearly cosmopolitan distribution, occurring throughout the warmer parts of the world. In Europe they are replaced by the temperature palaeartic wheat weevil (Plarre, 2010).

The adult female weevil bores in a grain, nut, or seed, and deposits an egg per individual grain. She seals the hole with a secretion. The larva develops while feeding on the interior of the grain, and then pupates. It usually leaves the grain completely hollow when it exists as adult (Plarre, 2010). The wheat weevil can live on acorns, and may have used them as a host before agriculture made grain plentiful. The rice weevil can live on beans, nuts, grains and some types of fruits such as grapes (Ebeling, 2011). Several other *Sitophilus* use the acorns of oaks such as bluejack oak (*Quercus incana*) and moru oak (*Quercus floribunda*). Some use the seeds of trees in dipterocarpaceae and the legume family, Fabaceae. The tamarind weevil (*Sitophilus linearis*) is only known from the seeds of tamarind (Plarre, 2010).

Several *Sitophilus* species are hosts to an intracellular γ -proteobacterium weevils and bacterium have a symbiotic relationship in which the bacterium produces nutrients such as amino acids and vitamins for the host supplementing its cereal diet (Vallier et al., 2009). As of 1993, there are about 14 species of *Sitophilus* (Ebeling, 2011). They include *Sitophilus conicollis*, *Sitophilus cribosus*, *Sitophilus erosa*, *Sitophilus glandium*, *S. granarius*, *S. linearis*, *S. oryzae*, *Sitophilus quadrinotatus*, *Sitophilus rugicollis*, *Sitophilus rugosus*, *Sitophilus sculpturatus*, *Sitophilus vateriaea*, *S. zeamais* (maize weevil) (Schoenherr, 1938).

Oryza sativa, commonly known as Asian rice, is the plant species most commonly referred to in English as rice. *O. sativa* is a grass with genome consisting of 430mb across 12 chromosomes. It is renowned for being easy to genetically modify, and is a model organism for cereal biology (Vallier et al., 2009).

Rice is a seed of the grass species *O. sativa* (Asian rice) or *Oryza glaberrima* (African rice). As a cereal grain, it is the most widely consumed staple food for a large part of the world's human population, especially in Asia. It is the agricultural commodity with the third highest worldwide production, after sugar cane and maize. Since a large portion of maize crops are grown for purposes other than human consumption, rice is the most important grain with regards to human's nutrition and caloric intake, providing more than one fifth of the calories consumed worldwide by humans (Ivbijaro, 1983).

Rice is a monocot that is normally grown as an annual plant, although in tropical areas it can survive as a perennial and can produce Raton crop for up to 30 years.

Rice cultivation is well-suited to countries and regions with low labor costs and high rainfall, as it is labor intensive to cultivate and requires ample water. However, rice can be grown practically anywhere, even on a steep hill or mountain area with the use of water –controlling terrace systems. Although its parent species are native of Asia and certain parts of Africa, centuries of trade and exportation have made it common place in many cultures worldwide (FAO, 2014).

Cereal crops especially rice is widely attacked by the grain weevil *Sitophilus* species causing 25 to 100% post-harvest losses in storage (Okonkwo, 1998). *S. zeamais* and *S. oryzae* are the two main species known to attack maize and rice in Nigeria. Owing to the insidious feeding habits, they are often undetected until damage has occurred (Okonkwo, 1998). Owing to the losses resulting from the feeding activities and damages of the rice grain pests, it is essential that necessary control measures are put in place to ensure adequate rice production and storage (Bekele and Hasanali, 1997). Insect pest in stored food products has relied heavily on the use of gaseous fumigants and residual contact insecticides. The implication of these are serious problems of toxic residues, health and environmental hazards, development of insect strain resistance to insecticides, increasing cost of application and erratic supply in developing countries due to foreign exchange constraints (Okonkwo and Ewete, 1999).

Similarly, Tetees and Gilstrap,(1998) were of the view that the use of chemical insecticides which hitherto was the control measure adopted in storage, has of recent been criticized because of the difficulties associated with its procurement, hazards with its use, development of pest resistance and pest resurgence problems. Thus, these problems have created the need to find materials that will effectively protect stored grains or produce, that are readily available, affordable, relatively less poisonous and less detrimental to the environment (Abd-el-Azc and Ismail, 2000).

Different spice and herbal plant products in the form of essential oil (EO), powders, pellets, extracts or distillates could be harnessed as potential toxicants, deterrents, antifeedants, repellents, and fumigants for exclusion of stored product pests from grain, have been used. *Xylopi aethiopica* is an evergreen, aromatic tree, of the Annonaceae family that can grow up to 20 m high. It is native to the lowland rainforest and moist fringe forests in the savanna zones of Africa. The dried fruits of *X. aethiopica* (Grains of selim) are used as a spice and as herbal medicine (Moller and Maier, 2010).

Xylopi a is a compression from Greek xylonpikron meaning "bitter wood". The second part of the plant's binomial name, *aethiopica*, refers to the origin of the tree, in Ethiopia, though currently it grows most prominently as a crop in Ghana (Harris et al., 2011). *X. aethiopica* grows in tropical Africa; It is present in the rain forests, especially near the coast. It also grows in the riverine and

fringing forests, and as a pioneer species in the savanna region. The wood is known to be resistant to termite attack and is used in hut construction; posts, scantlings, roof-ridges and joints. An infusion of the plant's bark or fruit has been useful in the treatment of bronchitis and dysenteric conditions, or as a mouthwash to treat toothaches. It has also been used as a medicine for biliousness and fibril pains. In Senegal, the fruit is used to flavor café Touba, a coffee drink that is the country's spiritual beverages and the traditional drink of the Mouride brotherhood (Harris et al., 2011). In the middle ages, the fruit was exported to Europe as a 'pepper'. It remains an important item of local trade throughout Africa as a spice, and flavoring for food and medicine. The fruit is sometimes put into jars of water for purification purposes (Burkil and Humphrey, 1985).

The African pepper or spice tree, *X. aethiopica* is an important, evergreen, medicinal plant widely distributed in West Africa, and concoctions prepared from its morphological parts are used in traditional medicine for the treatment of skin infections, candidiasis, cough, fever, dysentery and stomach ache (Okigbo et al., 2005). Extracts from *X. aethiopica* have been reported to exhibit antibacterial and antifungal (Okigbo et al., 2005) mosquito repellent (Adewoyin et al., 2006), and termite anti-feedant activities (Lajide et al., 1995). *X. aethiopica* can be harnessed in the form of oil extracts and powders for use in storage and entomology (Bekele and Hasanali, 1997). The achievement in this direction will help to increase the scope of rice production and utilization, to meet up with the ever increasing demand for rice and rice products (Okonkwo and Ewete, 1999).

Chemical pesticides has made way to controlling and management of pests both in field and in store, but the menace they cause is one that gives serious concern to man, his animals and the environment. As a result, scientists have been looking for a way to produce a more friendly pesticide that will not cause serious harm and so manageable; it is with this in mind that the zeal for the study was conceived.

Though works has been carried out by some authors with botanicals/biopesticides, the aqueous extract of the stem bark of *P. santalinoides* has been established to have effects on *Pseudomonas aeruginosa* (Eze et al., 2012). According to Anowi et al. (2012), the methanolic extract of leaves of some botanicals possesses analgesic activity. Okpo et al. (2011) reported the anti-diarrhea property of aqueous extract of some plant leaves. Other workers on biopesticides includes Adeleke et al. (2009) who reported the larvicidal properties of some plant extract; and Otitoju et al. (2014) who reported the welcoming effect of the plant in food. There is still paucity of information on the efficacy, effectiveness and potency of *X. aethiopica* as grain protectant against *S. oryzae* (Kunz et al., 2001).

This study investigated the efficacy, effectiveness and potency of *X. aethiopica* extracts (ethanolic and

aqueous), and evaluated the mortality, oviposition, developmental and natality rates of *X. aethiopica* in the control of *S. oryzae* on stored rice.

MATERIALS AND METHODS

Cereal grains

One kilogram of dry African rice *O. glaberrima* was collected from Ogige market, Nsukka, Enugu State and identified. The rice was fumigated for 48 h with carbon tetra chloride and aerated for 7 days in order to kill the resident insect pest as suggested by Ivbijaro (1983). The seeds were sieved with a 2-mm sieve to remove dead insects, and the processed grains were packaged in polythene bags and kept pending use (Becky, 2004).

Sitophilus oryzae

A weevil culture of *S. oryzae* that developed on rice was established on March 2015. The mass production of *S. oryzae* took place in the Applied Entomology Laboratory, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Enugu State, Nigeria with relative humidity and temperature of 66.6% and 28±5°C, respectively. Hundred adults of mixed sex (male and female) of *S. oryzae* were obtained from the weevil culture and reared in glass jar covered with muslin cloth. The food media were the rice. After two weeks, when oviposition has been noticed, the parent stocks of *S. oryzae* were removed by sieving the grain with a 2.00 mm sieve. The grains with the oviposited ova were left under laboratory conditions till emergence of F1 progeny. The F1 progenies from the cultures were used for the experiment (Ekeh et al., 2013).

Plant materials

The fruits of *X. aethiopica* were collected from Cemetery Market, Aba, Abia State and were dried to constant weight under room temperature. The dry material was ground to very fine powder and used for the experiment. The experiment was to find out the comparative efficacy of the seed extracts (Keay et al., 1964).

Aqueous extract of *Xylopia aethiopica*

Five hundred grams of the powdered fruits of *X. aethiopica* was ground and weighed into 1000-ml conical flask. 800 ml of distilled water was added to the sample in the flask. The solution was then stirred with a glass rod and allowed to soak for 24 h. The aqueous extract was filtered thrice through a plug of adsorbent cotton wool embedded in a glass funnel. The filtrate was then filtered through 11 cm round filter paper. The solution was concentrated by gentle evaporation on a heating mantle and poured into 500 ml beaker (AOAC, 2000).

Ethanolic extract of *Xylopia aethiopica*

Five hundred grams of the fruit was ground into fine powder and weighed using the electrical weighing balance. Five hundred milliliters of absolute ethanol was added to the sample in the flask. The solution was stirred with a glass rod, allowed to soak for 24 h, filtered using the filter paper and the filtrate allowed to evaporate under room temperature.

Table 1. Phytochemical screening of *Xylopi aethiopica*.

Parameter	Ethanolic extract	Aqueous extract
Alkaloids	+	++
Glycosides	++	+
Saponins	+	++
Tannins	-	-
Flavonoids	++	+
Reducing compounds	++	+
Polyphenols	++	+
Phlobatannins	-	-
Anthraquinones	-	-
Triterpenes	+	-
Steroids	+	-

-Not present; + present in small concentration; ++ present in moderately high concentration.

Table 2. Quantitative estimation of some phytochemicals of *Xylopi aethiopica*.

Parameter	Concentration (%)
Alkaloids	2.401±0.1
Flavonoids	8.10±0.1
Saponins	1.36±0.01
Polyphenol	2.75±0.03

Table 3. Percentage proximate analysis of *Oryza sativa*.

Nutrients	% Proximate analysis
Moisture	7.33 ± 0.1
Fat	0.50 ± 0.01
Crude protein	6.22 ± 0.01
Crude fibre	1.50 ± 0.10
Ash	1.00 ± 0.00
Carbohydrate	83.45 ± 0.1

Phytochemistry of plant materials

Phytochemical analysis was carried out for the presence of alkaloids, saponins, flavonoids, plobatannins, cardiac glycosides, tannins and anthraquinones (Table 1). These were screened using the method described by Trease and Evans (1996).

Quantitative estimation of some phytochemicals

Quantitative estimation of alkaloids, flavonoids, saponins, polyphenols and reducing compounds were determined using the method described by AOAC (1990) (Table 2).

Proximate study of rice (*Oryza sativa*)

The proximate study was carried out to ascertain the constituent nutrient (Carbohydrate, crude protein, ash, crude fibre, fat, moisture) and the level of their presence in rice grain following

the methods of Okoh and Ugwu (2011) (Table 3).

Experimental design

Split plot design of four concentrations replicated three times was used. Ethanollic and aqueous extracts of *X. aethiopica* was used for the experiment. Five pairs (five males and five females) of zero day old *S. oryzae* were introduced into each of the jars already with 20 g of rice and 8 g/6 g/3 g/0 g of *X. aethiopica* each. The ethanollic and aqueous extract was applied at the 8, 6 and 3 per 20 g of rice. Another control group was set up with the grain and *S. oryzae* but no botanical (experimental control). Each jar was covered with a muslin cloth to allow air movement and prevent insects from leaving the jar. The set up was allowed for 15 days with daily monitoring. Dead insects in each jar was collected and counted and the percentage insect mortality was calculated. When the eggs were noticed in the set-up, the pest *S. oryzae* was removed and eggs counted and recorded for each jar, the oviposition was monitored for 10 days for the inception of larva and pupa where developmental study was done. When the larva and pupa was noticed, the set up was monitored till natality of pests occurred and newly emerged adults of *S. oryzae* counted. The whole experiment lasted for a period of seven weeks (50 days) (Ekeh et al., 2013).

LD₅₀ of *Xylopi aethiopica*

Fifty percent lethal dose (LD₅₀) of *X. aethiopica* was done using the methods described by Don-Pedro (1989). The concentration of ethanollic and aqueous extract that killed 50% of the 20 *S. oryzae* exposed to it for a period of 24 h was recorded (Ousman et al., 2007).

Data analysis

One way analysis of variance (ANOVA) was carried out to compare differences in treatment means. Significant treatment means were separated using Duncan multiple range test. The efficacies of the ethanollic and aqueous extracts were also compared with Duncan multiple range tests.

RESULTS

Mortality

Table 4 presents univariate analysis of mortality rate on ethanol extract using analysis of variance (ANOVA). From the results shown, it was observed that the mortality of *S. oryzae* increased with increase in the dosage application. The highest mortality of weevil was recorded in 8 g (20.00 ± 0.58) of ethanollic extracts which was followed by 6 g (9.33 ± 0.67) and 3 g (3.33 ± 0.88). The least mortality was seen in 0 g (1.33 ± 0.33) due to the absence of the extract and it is the control group. There was no significant difference between 0 g and 3 g but there was significant difference between 6 g and 8 g.

Oviposition

Table 4 presented univariate analysis of oviposition rate

Table 4. Activities of *Xylopi*a *aethi*o*p*i*c*a against *Sitophilus oryzae*.

Concentration (g/20 g grain)	Ethanolic	Aqueous
Mortality		
Control (0.0)	1.33 ± 0.33 ^c	1.33 ± 0.33 ^c
3.0	3.33 ± 0.88 ^c	1.00 ± 0.00 ^c
6.0	9.33 ± 0.67 ^b	4.00 ± 1.55 ^b
8.0	20.00 ± 0.58 ^a	11.67 ± 0.88 ^a
Oviposition		
Control (0.0)	22.33 ± 1.45 ^a	22.33 ± 1.45 ^a
3.0	14.67 ± 0.88 ^b	17.67 ± 1.33 ^b
6.0	10.33 ± 0.88 ^c	17.67 ± 0.67 ^b
8.0	4.67 ± 0.33 ^d	8.33 ± 0.33 ^c
Emergence (Larva)		
Control (0.0)	21.67 ± 0.88 ^a	21.67 ± 0.88 ^a
3.0	13.00 ± 0.58 ^b	16.67 ± 0.88 ^b
6.0	8.33 ± 0.33 ^c	15.67 ± 0.67 ^b
8.0	3.67 ± 0.33 ^d	8.00 ± 0.00 ^c
Emergence (pupa)		
Control (0.0)	18.00 ± 1.15 ^a	20.67 ± 0.67 ^a
3.0	11.67 ± 0.67 ^b	15.33 ± 0.88 ^b
6.0	6.33 ± 0.67 ^c	12.33 ± 0.33 ^c
8.0	2.33 ± 0.67 ^d	6.33 ± 0.33 ^d
Natality		
Control (0.0)	16.33 ± 0.88 ^a	19.67 ± 0.88 ^a
3.0	11.00 ± 1.00 ^b	14.00 ± 0.58 ^b
6.0	4.67 ± 0.88 ^c	11.33 ± 0.33 ^c
8.0	1.33 ± 0.67 ^d	4.67 ± 0.33 ^d

Values with different alphabet superscript for a parameter in a column are significantly different ($p < 0.05$).

on the ethanolic and aqueous extracts of *X. aethi*o*p*i*c*a using analysis of variance (ANOVA). From the study, it was observed that *S. oryzae* were able to lay eggs at all treatment dosages. However, the oviposition rate of the insects decreased with increase in the dosage application. The highest oviposition was observed in 0 g (22.33 ± 1.45) for both extracts, which was followed by 3 g (14.67 ± 0.88) and 6 g (10.33 ± 0.88). The least oviposition was observed in 8 g (4.66 ± 0.33), (8.33 ± 0.33) for both extracts which has the highest concentration of the extract. There was significant difference in all the concentrations going by the superscript of performance of ethanolic and aqueous extraction methods of *Xylopi*a *aethi*o*p*i*c*a.

Figure 1 compares the performance of ethanolic and aqueous extraction methods of *Xylopi*a *aethi*o*p*i*c*a. From all indications, ethanolic extraction was more efficacious than aqueous.

Developmental rate (larva)

Table 4 presents univariate analysis of developmental rate (larva) on the ethanolic extract using analysis of variance (ANOVA). The result of the bioactivities of *X. aethi*o*p*i*c*a ethanolic extract on the larval stage of *S. oryzae* as presented in Table 4 shows that more larva were observed in the control, followed by 3 g, 6 g and the lowest number of larva was observed on the 8 g for both ethanolic and aqueous extraction due to the concentrations. There was significant difference in all the concentrations going by the superscript.

Developmental rate (Pupa)

Table 4 presents univariate analysis of developmental rate (pupa) on the ethanolic and aqueous extract using analysis of variance (ANOVA). The result of the bioactivities of *X. aethi*o*p*i*c*a extracts on the larval stage of *S. oryzae* as presented in Table 4 shows that more pupa were observed in the control, followed by 3 g, 6 g and the lowest number of pupa was observed on the 8 g due to the effect of the extracts and concentration. Going by the superscript, there were significant differences in all the concentrations.

Natality

In Table 4, the result showed that the natality of *S. oryzae* decreased with increased dosage application. The untreated grain 0 g (16.33 ± 0.88) has the highest rate of natality for both ethanolic and aqueous extractions which was followed by 3 g and 6 g. The least birth was observed in 8 g (1.33 ± 0.67), (4.67 ± 0.33) which shows the lowest number of natality. There was significant difference in all concentration going by the superscript.

DISCUSSION

In the present study, the ethanol and aqueous extracts prepared from *X. aethi*o*p*i*c*a have shown very high insecticidal properties against *S. oryzae*. The ethanolic and aqueous extracts of *X. aethi*o*p*i*c*a at the dosage rates of 3, 6 and 8 g in rice gave promising levels of control of *S. oryzae* in terms of increase in mortality and reduction in oviposition, natality and developmental rates with the ethanolic extracts having high insecticidal properties than the aqueous. The present work agreed with the findings of Yankachi and Gadachi (2010) where the insecticidal actions of different plant products were compared against *S. oryzae*. Onolemhemhen and Oigiangbe (1991) have also reported the superiority of *X. aethi*o*p*i*c*a and *Piper guinensis* seed in increasing mortality against *S. oryzae*. Onolemhemhen et al. (2011)

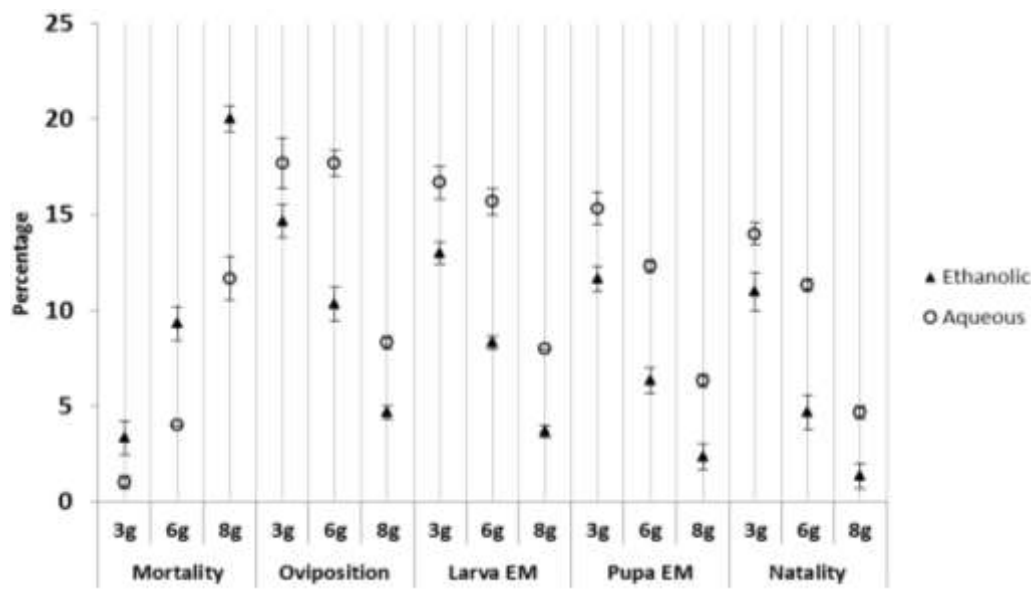


Figure 1. Comparison of activities of the two extraction solvents. Activity of ethanolic extract significantly better than aqueous extract at inhibition of oviposition, larva and pupa emergence and natality ($p < 0.05$). Ethanolic extract is also significantly more effective at causing mortality ($p < 0.05$).

reported that *X. aethiopica* has a pungent smell which functioned more as an insect repellent and perhaps caused suffocation and death of the weevils. The insecticidal features of the plant's extract may be due to their constituent components, pungent smell and mode of actions. These may have accounted for their effective control of *S. oryzae* and other insects. Extracts of *X. aethiopica* might have exhibited some insecticidal actions resulting in low natality and oviposition of *S. oryzae* even though the natality and oviposition of *Sitophilus oryzae* were not completely halted at any of the treatment dosage rates (8 g, 6 g, 3 g), they were lower than the control treatment (0 g). This agreed with the findings of Edelduok et al. (2015) that cotyledon powder of melon halted the oviposition of *S. zeamais* which might have been due to the presence of essential oil. The mode of action of oil has been suggested by Credland (1992) to include physical barrier to respiration of insect's eggs and young larvae. Moreover, Rajapaske (2006) suggested that the mechanical effects of the large quantities of powders could have effects on oviposition. Lubijar (1983), who embarked on another botanical study of the neem seed *Azadirachta indica* found out that the neem seed severely reduced egg laying in female *S. oryzae* while increasing the mortality (Ekeh et al., 2013).

Treatment with *X. aethiopica* increased larval and pupal period and reduced the total oviposition period, adult longevity and fecundity suggesting that the phytochemicals in the *X. aethiopica* interfere with the neuroendocrine system in insects, which controls the synthesis of ecdysone and juvenile hormone. In the present investigation, it was found that *X. aethiopica*

affords better protection against *S. oryzae*. It was observed that the ethanolic extract was more effective in checking mortality, natality, developmental rate and oviposition than the aqueous extracts. *X. aethiopica* has many other activities against insects disrupting or inhibiting development of eggs, larvae, pupae, delaying the molting of larvae, disrupting mating and sexual communication, repelling larvae and adults, poisoning larvae and adults, feeding deterrent and preventing adult to maturation. This is in agreement with the work of Radha and Susheela (2014) who reported that the use of different botanicals severely reduced the oviposition, developmental rates and natality of *Callosobruchus maculatus*. The findings of this work indicated that the ethanol extract of *X. aethiopica* could be used in controlling rice weevil in stored rice. This will reduce chemical pesticide usage, remove the risk of toxic residues in food and ensure the continued availability of insect free rice for food, planting, trading and storage (Ngamo et al., 2007).

Conclusion

The present work has revealed the efficacy of *X. aethiopica* on rice weevil (*S. oryzae*), established the concentration that controls the weevils on rice grain at different quantities and has also looked at different extraction methods of the *X. aethiopica*. The effects of *X. aethiopica* in protecting the grain against *S. oryzae* were comparable to using standard pesticides. It has been confirmed that ethanolic extraction stands out as the best

extraction methods because it exposes the active ingredients of the extract making it more efficacious as having the potentials in the control of insect pests and thus recommended as a biopesticide.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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