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COMPARATIVE STUDY OF THE TOXICITY OF OILS FROM SEEDS OF *Citrullus colocynthis* AND *Citrullus vulgaris* ON LARVAE OF *Dermestes Maculatus*

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Abstract:

Study was carried out in the Fisheries Research Laboratory, Ahmadu Bello University to evaluate the effect of *Citrullus colocynthis* (Eguisi) and *Citrullus vulgaris* (Watermelon) seed oils on the larvae of *Dermestes maculatus*, an important pest of smoke-dried *Clarias gariepinus* (African catfish). The effect of the oils at different tested concentrations against the larvae of *D. maculatus* was dose dependent as 3.33% and 81.11% mortality for *C. colocynthis* while 2.22% and 91.11% for *C. vulgaris* were recorded for 0.027 mLg^{-1} and 0.081mlg^{-1}respectively at 96 hours' exposure time. The interaction effect of 0.081mlg^{-1} watermelon seed oil and 96h exposure gave the highest kill compared to eguisi seed oil at the same concentration and time which was significantly ($p<0.05$) more than the percent kill of the other concentrations and exposure time. At 0.243 mLg^{-1} both oils killed 100% of *D. maculatus* larvae even at 24h exposure time. It is therefore concluded that 0.081mlg^{-1} and 0.243 mLg^{-1} of both *C. colocynthis* and *C. vulgaris* seed oil could be applied as botanical insecticides to prevent smoke-dried fish from *D. maculatus* larvae attack.

Keywords: *Citrullus colocynthis*, *Citrullus vulgaris*, *Dermestes maculatus* larvae, Larvicidal effect, Dried catfish

Introduction

Citrullus vulgaris and *Citrullus colocynthis* belongs to a large plant family called the Cucurbitaceae known for its great diversity and widespread adaptation in tropical and subtropical regions, arid deserts and temperate locations (Oluba, Adeyemi, Ojieh & Isiosio, 2008). It consists of nearly 100 genera and 750 species, known for their high protein and oil content. Seeds of curcubits are sources of oils and protein with about 50% oil and up to 35% protein (Achu, Fokou, Tchiégang, Fotso & Tchouanguep, 2005). Eguisi (*Citrullus colocynthis* L.) and watermelon (*Citrullus vulgaris* L.) belongs to the species of the genus *Citrullus* of curcubitaceae family, which usually consists of a large number of varieties that are generally known as melons (Mabaleha, Mitei & Yeboah, 2007). Seenivasan, Jayakumar, Raja & Ignacimuthu (2004) reported that *Citrullus colocynthis* showed highest repellent activity in lower concentration against *Callosobrochus maculatus*. Akpotu & Adebote (2013) reported that 1.38ml of *C. colocynthis* oil applied on 17g *Clarias gariepinus* dried fish gave 44% repellent protection while the same concentration of *C. vulgaris* seed oil gave a much better repellence (71.11%). Watermelon seeds have been observed to be mildly diuretic and its consumption may have antihypertensive effect (CBC News site, 2008) while its eguisi counterpart is heavily consumed for the food in the seeds and used both as condiment and thickener in various Nigerian local soups (Uruakpa & Aluko, 2004).

Stored products like grains, cheese, hide, fur, bacon, dried fish, meat, and other protein-containing concentrates, have been known to be destroyed by insect pests. Insect infestation of cured fish by blowflies and hide beetles is an important cause of post – harvest losses in many developing countries (Johnson & Esser, 2000). Fish is susceptible to attack by insect pests throughout processing and storage. The principal pests are blowflies (Diptera: Calliphoridae and Sarcophagidae) and hide beetles (Coleoptera: Dermestidae and Cleridae). Losses caused by infestation could be physical, economical and nutritional in nature (Johnson & Esser, 2000). According to Osuji (1974); Eyo & Awoyemi (1989), large scale deterioration in quality and quantity of dried fish is attributed to dermestid infestation. Prominent insecticide families Organochlorine hydrocarbons (e.g. DDT) have been used in the control of pest of stored products but they have been phased out because of their persistence and potential to bioaccumulate (Kamrin, 1997). They operate by disrupting the sodium/potassium balance of the nerve fibre, forcing the nerve to transmit continuously.

Unlike synthetic chemical insecticides that kill both pests and non – target organisms, natural insecticides including botanicals are relatively target specific (Isman, 1997). Plant materials such as spices, vegetable oils, extracts, powder or ash (Keita, Vincent, Schmit, Arnason & Bélanger, 2001; Akinkurolere, Adedire & Odeyemi, 2006; Adedire, Obembe, Akinkurolere & Oduleye, 2011) have been reported for their insecticidal efficacy. *Dermestes maculatus* is an important pest of dried fish and meat in many regions of the world (Integrated Information System, 2009). A comparative assessment of the biological performance of *D. maculatus* in various dietary media namely dried fish, fish meal, bone meal, palm kernel meal, blood meal and whole meal revealed that dried fish followed by fish meal were significantly superior to the commercial feeds (Osuji, 1978). Management of agricultural pests over the past half century has been largely dependent on the use of synthetic chemical pesticides both for field and post-harvest protection of stored products. Potential problems associated with continued long term use of toxic insecticides include pest resistance and negative impact on natural enemies (Abudulai, Shepard & Mitchell, 2001). For this reason, plants and their products are exploited for their benefits as possible control agents against pests of stored products, in this case *D. maculatus* larvae. Researchers have begun to assess plant essential oils as alternatives to fumigants and contact insecticides (Isman, 2000; Wang, Tasi, Ding, Zhao & Li, 2001).

The objective of the present study was therefore to determine the larvicidal effect of the oils on the late instar larvae of *D. maculatus* and compare their effects on the pest.

Materials and Methods

Culture of *Dermestes maculatus*

Adult *D. maculatus* was obtained from infested fishes at Sabon gari market in Zaria, Kaduna state, Nigeria. The beetles were reared in clean kilner jars containing whole and fragmented fishes. The jars were capped with muslin cloth and kept at ambient temperature (27 ±3ºC) and rela-

tive humidity of 75 ±%. The muslin cloth allowed for ventilation and also prevented entry or exit of beetles and other insects. The beetles were allowed for 5 days to oviposit on the fishes. At the end of five days, the beetles were removed by hand picking and added to another sterilized jar of fish to raise new generations of *D. maculatus* larvae. The culture was then maintained by continually replacing the devoured and infested fishes with fresh disinfested ones.

Collection and Processing of Plant materials

The seeds of *C. colocynthis* and *C. vulgaris* were purchased from seed marchants in Sabon gari market, Zaria. They were air-dried for three (3) days in the shade. The dried seeds were then pulverized into powder using mortar and pestle. The powder was put in cellophane bags and kept until needed.

Oil Extraction

40g of each powder was extracted using n-hexane with the help of a soxhlet apparatus. The extract was then transferred to a water bath to separate the solvent from the oil. The extracted seed oils from *C. colocynthis* and *C. vulgaris* were stored in separate labeled bottles and kept in a cool place until used in bioassay.

Bioassay

The smoke-dried fishes for the experiment were heat sterilized in the oven set at 60 ±2ºC for an hour and then allowed to cool. After cooling to room temperature, each fish was weighed and tagged. 0.003 mLg^{-1}, 0.009 mLg^{-1}, 0.027 mLg^{-1}, 0.081 mLg^{-1} and 0.234 mLg^{-1} crude seed oil of *C. colocynthis* and *C. vulgaris* were applied to the whole fish and placed in kilner jars. The toxicity of the seed oils was recorded after 24, 48, 72 and 96 hours. All the treatments including control were replicated three times and data collected were analyzed statistically at $p<0.05$. One Way Analyses of variance (ANOVA) was used to determine if there is significant difference between the various treatments and where differences exist means were separated by Duncan's multiple range test (DMRT). Probit Analysis was also used to determine the 96 hour LC_{50} of the seed oils.

Results and Discussion

The mortality result of this experiment is presented in Table 1 and 2.

Table 1. Mortality effect of *Citrullus colocynthis* seed oil on *Dermestes maculatus* Larvae in 96 hours Exposure time

Seed oil conc.	Exposure Time in hours				
	24h	48h	72h	96h	**P-Value**
Control	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}	**0.79**
0.003mlg^{-1}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}	**0.79**
0.009mlg^{-1}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}	1.11±0.33^{d}	**0.47**
0.027mlg^{-1}	2.22±0.67^{c}	2.22±0.67^{c}	2.22±0.67^{c}	3.33±0.58^{c}	**0.46**
0.081mlg-1	71.11±4.18^{b}	74.44±4.70^{b}	76.67±5.03^{b}	81.11±3.71^{b}	**0.06**
0.243mlg^{-1}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	**0.97**
P-Value	**0.00**	**0.00**	**0.00**	**0.00**	

Mean ±SEM with same superscript within columns are not significantly different from each other at $p<0.05$

Table 2. Mortality effect of *Citrullus vulgaris* seed oil on *Dermestes maculatus* Larvae in 96 hours Exposure time

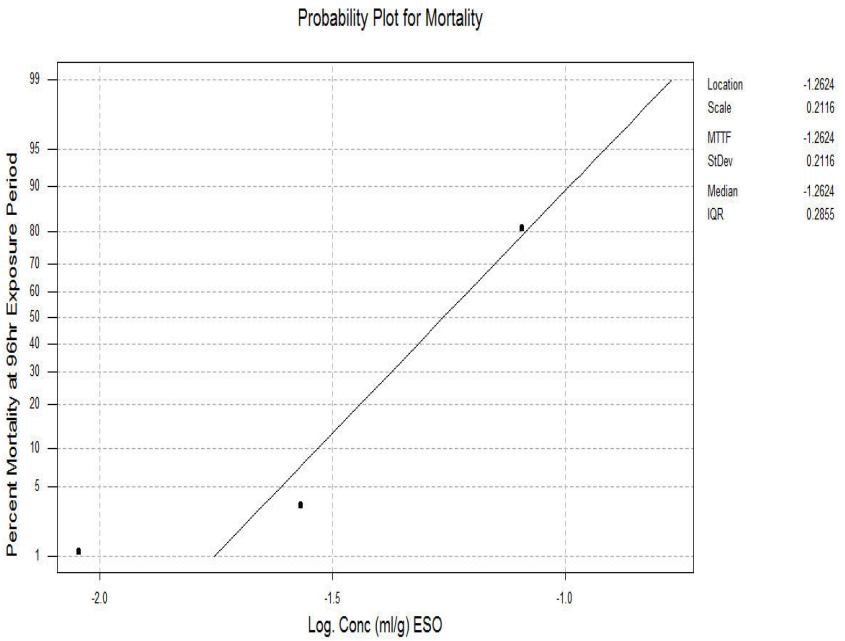
Seed oil conc.	Exposure Time in hours				
	24h	48h	72h	96h	**P-Value**
Control	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}	**0.79**
0.003mlg^{-1}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}	**0.79**
0.009mlg^{-1}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}	**0.79**
0.027mlg^{-1}	1.11±0.33^{c}	2.22±0.33^{c}	2.22±0.33^{c}	2.22±0.33^{c}	**0.01**
0.081mlg-1	78.89±1.45^{b}	86.67±0.58^{b}	87.78±0.88^{b}	91.11±0.88^{b}	**0.19**
0.243mlg^{-1}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	**0.97**
P-Value	**0.00**	**0.00**	**0.00**	**0.00**	

Mean±SEM with same superscript within columns are not significantly different from each other at $p<0.05$

Table 1 and 2 showed that *C. colocynthis* and *C. vulgaris* respectively at 0.243 mLg^{-1} concentration exhibited the highest efficacy against *D. maculatus* followed by 0.081 mLg^{-1}, 0.027 mLg^{-1}, 0.009 mLg^{-1} and 0.003 mLg^{-1}. This implies that the toxic effect of *C. colocynthis* and *C. vulgaris* seed oils against the test larvae were dose dependent and significantly different ($p<0.05$) from each other. There was no significant difference ($p<0.05$) in the toxicity performance of both oils at 0.027 mLg^{-1} in all time frames considered in this study but there was clear significant difference ($p<0.05$) at 0.081 mLg^{-1} in all the time frame implying that C. vulgaris oil is more effective than oil of *C. colocynthis* in killing *D. maculatus* larvae.

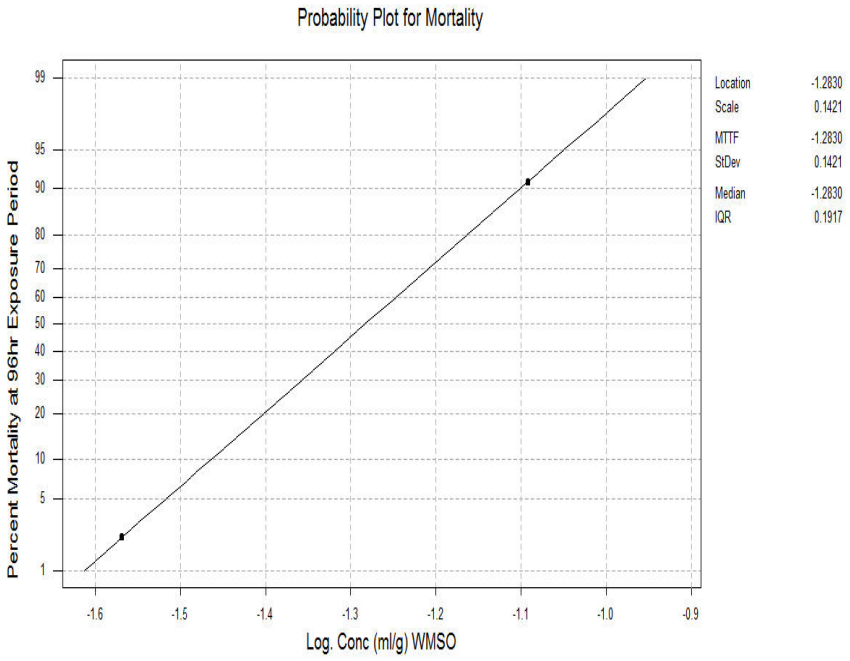
The LC_{50} (Figure 2.) of *C. vulgaris* at 24, 48, 72 and 96h exposure time was lower than that of *C. colocynthis* (Figure 1), implying that *C. vulgaris* seed oil was more effective than oil of *C. colocynthis* on *D. maculatus* larvae. The highest total mortality percentage (100%) was recorded at 0.243mLg^{-1} for both oils. The result also showed that there was positive interaction effect between treatments and exposure time but was also dose dependent. No mortality was observed in the control. The interaction effect of *C. colocynthis* oil at 0.081mLg^{-1} concentration for 72h showed that the 76.67% kill of the test insects was significantly more than that of 24, 48 and 72h at the same concentration but was significantly lower than that recorded for *C. vulgaris* oil which gave 87.78% kill at 72h and the same concentration.

The mortality of *D. maculatus* larvae caused by oils of *C. colocynthis* and *C. vulgaris* may be due to the effect of sterols and fatty acids on the cuticle of the insect or it may be due to the disturbance of hormonal regulations caused by sterols. This report is comparable to that of Kamel (2010) who attributed mortality of larvae and pupae of the Armyworm (*Spodoptera frugiperda*) to the whole components found in moringa oil. Ajayi (1929) also showed that the active compounds responsible for mortality of the insects are embedded in plant extracts. Asawalam, Emosairue, & Wokocha (2007) holds the view that insecticidal activity of any plant extract depends on the active constituents of the plant. These components could have worked synergistically to produce the mortality effect observed in this study.



ESO: Eguisi Seed Oil (*Citrullus colocynthis*)

Figure 1. Probit graph use to determine the LC_{50} of *Citrullus colocynthis* seed oil against *Dermestes maculatus* larvae



WMSO: Watermelon Seed Oil (*Citrullus vulgaris*)

Figure 2. Probit graph use to determine the LC_{50} of *Citrullus vulgaris* seed oil against *Dermestes maculatus* larvae

The result obtained in this study agrees with EL Nadi, EL Hag, Zaitoon & AL Doghairi (2001) who found that *Azadiracta indica* extract show a remarkable toxicity against *Trogoderma granarium* and that this toxic effect was found to be dose and exposure time dependent. Although *C. colocynthis* was comparatively less toxic than *C. vulgaris* seed oil against *D. maculatus* larvae, it was significantly more larvicidal to *D. maculatus* at all levels of concentrations compared to the control. This agrees with the report of Nadeem, Iqbal, Khattak & Shahzad (2012) who holds a similar view. Since most insects breathe through the use of spiracles, the high larval mortality recorded in this experiment could be as a result of blockage of spiracles or air chamber of the beetles causing death by suffocation. This agrees with Don-pedro (1989) who holds a similar view.

Conclusion

On the basis of results, it can be concluded that *C. colocynthis* and *C. vulgaris* are good control agents of *D. maculatus* larvae and are most effective at 0.243 mLg^{-1}. The larvicidal effects of both oils are dose and time dependent. Seed oil extract of *C. vulgaris* was a superior larvicide to *C. colocynthis* seed oil. It is therefore recommended that *C. colocynthis* and *C. vulgaris* at 0.081 mLg^{-1} and 0.243 mLg^{-1} could be utilized in the management of *D. maculatus* larvae in smoke-dried fish stores.

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