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Bio-efficacy of some plant oil extracts as surface protectant against leather beetle, *Dermestes maculatus* (De geer) on smoked fish of *Clarias gariepinus*

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Abstract
The oil extract of *Corchorus olitorius*, *Solanum nigrum*, *Lycopersicon esculentum* and *Telferia occidentalis* were applied respectively at concentrations of 0, 2%, 4%, 6% and 8% to the disinfected muscle of smoked *Clarias gariepinus*. Forty four adults (22 males and 22 females) of newly emerged *Dermestes maculatus* (0-24hrs old) were introduced into the plastic plates, each containing 40grams of treated and untreated smoked catfish. The plastic plates were covered immediately with muslin cloth to prevent the insect from escaping and ensuring aeration. Insect infected smoked catfish, *C. gariepinus* in plastic plates without any of the oil extracts served as control. Each treatment was replicated thrice and laid out in a completely randomized design. Evaluation of the potency of the oil extracts was based on the adult insect mortality, percentage egg hatchability, death of larvae and pupae formation. The weight loss in fish muscle in the treated and untreated samples were compared as index of fish damage during storage. The results showed that oil extract of *T. occidentalis, C. olitorius, and S. nigrum* were significantly (p<0.05) effective in killing all the adults, eggs, larvae and pupae of *D. maculatus* while the effect of oil extract from *L. esculentum* were not significantly different (p>0.05) from the untreated (control). The percentage weight loss in fish during storage shows a significant increase (p<0.05) in fish treated with the plant oil extract from *L. esculentum* and the untreated (control) fish compare with the fish treated with oil extract from *T. occidentalis, C. olitorius, S. nigrum.* This study revealed that oil extract from *T. occidentalis, C. olitorius* and *S. nigrum* were capable of controlling the different stages of *D. maculatus* in smoked fish (*Clarias gariepinus)* during storage.

Keywords: Plant, oil extract, leather beetles, smoked fish, *Clarias gariepinus*

1. Introduction
In Nigeria, more than 50% of smoked dried fish products are destroyed by insect pests during storage. Synthetic insecticides in most times is applied on the dried fish to retard spoilage which results in health hazards to most of the consumers and the environment. Fish serves as one of the major and cheapest source of animal protein and has been used steadily due to its availability and nutritional values; fish is also used to correct protein deficiency in human diets in the tropic area. With the high protein content, fish serves the purpose of natural supplement for red meat and cereals in the human diet. The consumption of fish provides essential nutrients to a great number of people globally and thus its significant contribution to nutrition cannot be over-emphasized, a decline in fish availability will have a detrimental effect on the nutritional status in places where fish contributes significantly to the protein intake of the people [1]. However, fishes are very perishable commodity compare to cattle, sheep, poultry and the decay/spoilage process sets in easily even in temperate climates; therefore they must be preserved immediately after capture. The African catfish, *Clarias gariepinus* is the most popular, greatly cultivated and mostly smoked fish. In Nigeria, *Dermestes maculatus* (De geer) have been reported to be a pre-dominant pest of dried fish and is recognized mostly as a cosmopolitan pest of stored items especially those containing animal protein. Losses in quantity and quality of dried fish destined for both human consumption and economic purposes during storage have been attributed to *Dermestes maculatus* infestation [2]. This pest accounts for about 71.5% of dried fish infestation in most of the producing areas with a substantial loss in dry weight of about 43-67% from both larvae and adults [1]. Some fish parasites may be transmitted to man through consuming raw, lightly-preserved or undercooked

infected fish [3, 4] In Nigeria we consume a lot of fish and offers the largest market for fish and fisheries products in Africa. Freezing of dried fish at a temperature of 6-12˚C to kill all developing stages of insects is often practiced, the only limitation to this fact is the unstable and unreliable power supply which makes the use of refrigerator undependable especially in poor and rural communities. Also the use of Primiphos-methyl on dried fish, leave harmful residue on the fish when not properly applied. Hence, the use of Plants derived oil extract which are more readily biodegradable, less toxic to mammals, easy and much more affordable to produce by local farmers and less likely to be resisted by an insect is highly recommended [5]. When dried fish was mixed with leaf, bark, seed powder, or oil extracts of selected plants, there was a mortality of beetles reduced oviposition rate, suppression of adult emergence and reduced fish damage rate [2]. These study was design to determine the efficacy of four plant extracts namely; *Telferia occidentalis, Solanum nigrum, Corchorus olitorius, Lycopersicon esculentum* as surface protectant against *Dermestes maculatus* as well as create an understanding of insect pest management in order to reduce dependence on the use of synthetic chemicals.

2. Materials and Methods

2.1. Study/Experimental Site

Calabar is the Capital of Cross River State, Nigeria. Its lies between Latitude $4^0$56'59.99''N and Longitude $8^0$19'18.00''E. Calabar is often described as the tourism capital of Nigeria. Administratively, the city is divided in Calabar municipal and Calabar south Local Government Areas. It has an area of 406 square kilometers and a population of 371,022 as at 2006 census. The city has an international museum, a botanical garden, a free trade zone/port, an international airport and seaport, an intergrated sport stadium complex, cultural centre, one of the most prominent University in the country - University of Calabar, a slavery history park and several historical and cultural landmarks. The temperature is relatively constant throughout the year, with average high temperatures usually ranging from 25 to 28(^{0}C). Calabar averages just under 3,000millimeter of precipitation annually. The experiment was conducted at the Department of Zoology and Environmental Biology laboratory, Faculty of Biological Sciences, University of Calabar at ambient temperature of 27±30 ˚C with relative humidity of 71% and twelve hours of day light.

2.2. Collection of Experimental Materials

2.2.1. Sources of Plant Materials

Corchorus olitorius (Jute leaf), *Telferia occidentalis* (Fluted pumpkin), *Solanum nigrum* (Garden egg leaf) and *Lycopersicon esculentum* (Tomato leaf) were obtained from the Mariam market in Calabar Metropolis, Cross River State and were taken to the Department of Botany for proper identification (Table 1).

Table 1: Plant names, uses and parts used for oil extraction and treatment of dry *C. gariepinus*

Scientific name	Common name	Family name	Uses	Parts of plant used for oil extraction
Telferia occidentalis	Fluted pumpkin, fluted gourd, Ogu, Ikong-ubong in Igbo and Efik respectively	*Cucurbitaceae*	In soup culinary and herbal use.	Leaves
Solanum nigrum	Black night shade	*Solanaceae*	Eaten as food, spices or medicinal.	Leaves
Lycopersicon esculentum	Tomato vines	*Solanaceae* (night shade).	Herbal tea at the right amount.	Leaves
Corchorus olitorius	Nalta jute, tossa jute, West Africa sorrel, bush okra and Jew's mallow.	*Malvaceae*	Vegetable, tea and soup thickener.	Leaves

2.2.2. Insect source and culture

Adults of *Dermestes maculatus* were obtained from heavily infested smoked-dried fish, purchased from Watt market, Calabar and were identified using taxonomic keys. The identified adults were introduced in plastic plates containing dried fish and immediately covered with a muslin cloth, to prevent the insects from escaping and ensuring aeration at room temperature and 70-80% relative humidity. The culture plastic plates were kept on laboratory shelf for *Dermestes maculatus* production. These reared insects (*Dermestes maculatus*) provided a stock of *Dermestes maculatus* for experimentation.

2.3. Collection of smoke-Dried Fish

Smoked-dried *Clarias gariepinus* were purchased from Watt market, Calabar for the experiment.

2.4. Processing of plant materials

Leaves of *Corchorus olitorius, Telferia occidentalis, Solanum nigrum* and *Lycopersicon esculentum* were dried in a laboratory using an electric oven at 40˚C for 5hrs, thereafter, it was ground to a fine powder using National (Model J657 HAAN) electric grinder. The resulting powder was sieved using 2mm mesh size and stored in pre-labelled clean plastic bags prior to oil extraction.

2.5. Soxhlet extraction of oils

100g of each plant leaf powder in muslin cloth was introduced separately into the Soxhlet chamber for oil extraction. In the round-bottom flask, 350ml of n-Hexane introduced as solvent. The extraction was done at 60-80˚C until the solvent in the Soxhlet chamber became transparent. The extract was filtered using Whatman No.1 filter paper, cooled in a desiccator and the resulting filtrate was kept in a deep freezer at -5˚C prior to use. A rotary evaporator was used to evaporate the excess solvent from the oil. Extracted plant oils were stored in separate labelled bottles until required for bioassay [6].

2.6. Effect of plants extracts on mortality of adult *Dermestes maculatus*

Each plant extract at concentration 0mg, 2.0mg, 4.0mg, 6.0mg and 8.0mg were applied separately to 40g of disinfected muscle of smoked *Clarias gariepinus* in plastic plates. Forty four adults (22 males and 22 females) of newly emerged *Dermestes maculatus* (0 – 24hrs old) were introduced into each plastic plate containing the treated and untreated smoked fish. The plastic plates were covered immediately with muslin cloth to prevent the insects from escaping and ensuring aeration. Insects infected smoked fish in plastic plates without any of the oil extract served as control. The experimental units were in triplicates and carried out in a complete

randomized design at ambient temperature (27±30˚C) with relative humidity at 75%. Insect mortality was monitored daily for the first 7 days after treatment and the percentage mortality was determined.

2.7. Effect of plants extracts on egg hatchability and larvae survival

To determine the effects of oil extract on egg hatchability of *D. maculatus* during storage, 0mg, 2.0mg, 4.0mg, 6.0mg and 8.0mg of each of the plant oil extract was added individually to 40g of minced muscle of smoked fish in glass petri dishes and thoroughly mixed with the aid of a glass rod. The fish muscles were air dried for 1-2 hour after which 40 newly laid eggs (about 24 hours old) of *D. maculatus* were introduced into each petri dishes and then covered. Egg infected fish muscle without application of any of the oil extract serve as control. Each treatment was replicated four times and kept in an open air shelf in the laboratory at room temperature and pressure. Number of larvae emerging was recounted at 48, 96, 144 and 192hrs after treatment. % hatchability was determined.

2.8. Effect of Plants extracts on larvae of *D. maculatus*

2.0mg, 4mg, 6mg and 8mg of each plant extract was introduced into mixed properly with 40gram of disinfested muscles of dry *C. gariepinus* in plastic plates. Twenty larvae of *D. maculatus* were introduced into each plate containing treated fish muscle. A plate containing untreated fish muscle and *C. gariepinus* larvae serve as control. Each experimental unit had four replicates land out in a completely randomized design under prevailing environmental conditions. Data was taken on number of death larvae at 24, 48, 72 and 96hrs after treatment and number of pupae developed at 40 days after treatment.

2.9. Biological Evaluation

The biological evaluation of oil extracts from plant leaves was based on the mortality of adults, egg hatchability, larval mortality/pupae formation after treatment and the percentage weight loss after 60 days of storage calculated as the difference between the initial and final weight of fish divided by initial weight and multiplied by 100.

2.10. Statistical Analysis

Data obtained were subjected Descriptive statistics such as Mean, Standard error. Analysis of variance (ANOVA) were used to check for significance difference within the plant extracts and was measured at 0.05 significance levels, the treatments mean were separated using least significance difference. Graphical illustration was done using Microsoft excel 2007.

3. Results

The effects of plant oil from leaves of *Telferia occidentalis* (To), *Solanum nigrum* (Sn), *Corchorus olitorius* (Co) *and Lycopersicon esculentum* (Le) on mortality of adults *D. maculatus* in smoked *Clarias gariepinus* is shown in Fig 1. The highest significant (p<0.05) percentage mortality was obtained from *C. gariepinus* treated with 6mg (70.5%) and 8mg (88.6%) of *T. occidentalis* and 8mg (72.7%) of *S. nigrum*. This was closely followed by treatment groups of 4mg of *T. occidentalis* (45.5%), 6mg of *S. nigrum* (52.8%) and 6mg and 8mg of *C. olitorus.* 6mg (35.2%) and 8mg (35.2%) of *L. esculentum* and 4mg (36.4%) of *C. olitorus* had comparable effects on *C. gariepinus*. The lowest and insignificant percentage mortality was obtained from 2mg (16.5%) of *C. oliterus*, 2mg (14.8%) of *L. esculentum* and the control (Fig. 1 and Table 2).

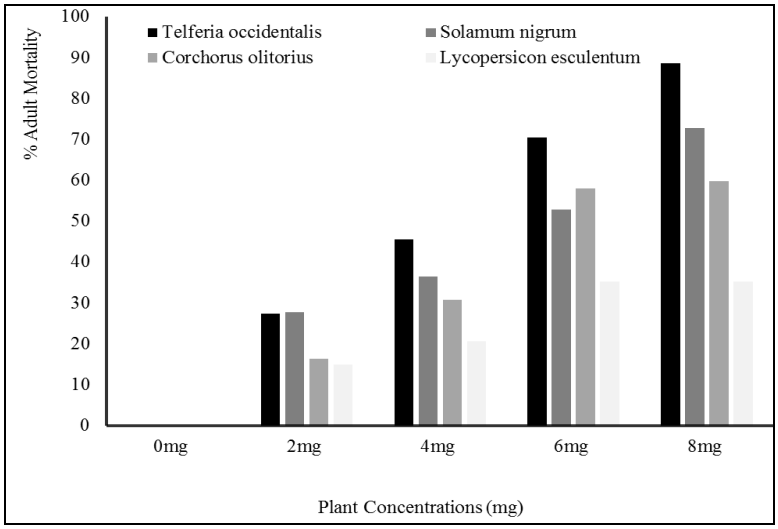

Fig 1: Effects of Plant Extract on adults mortality of *D. maculatus*.

Table 2: Effect of plant oil extracts on adults, pupae, larvae and egg stage of *Dermestes maculatus* on smoked dried *Clarias gariepinus*

Type of plants	Adult mortality (%)	Number of pupae formed (%)	Surviving Larvae (%)	Egg hatchability (%)
T. occidentalis	100 ± 0.00^{a}	0.00 ± 0.00^{a}	100 ± 0.00^{a}	0.00 ± 0.00^{a}
S. nigrum	100 ± 0.00^{a}	10.00 ± 0.00^{a}	1000.00^{d}	0.00 ± 0.00^{a}
L. esculentum	60.0 ± 1.53^{b}	20.0 ± 1.15^{b}	40.00 ± 0.67^{b}	36.70 ± 0.33^{b}
C. olitorius	100 ± 0.00^{a}	0.00 ± 0.00^{a}	66.67 ± 3.3^{a}	0.00 ± 0.00^{a}
Control	16.7 ± 0.33^{c}	63.30 ± 0.67^{c}	16.67 ± 0.33^{a}	86.67 ± 0.33

Each value is a mean of the triplicate samples (±SE). Mean followed by the same superscript(s) in the same column are not significant different (p>0.05).

The effect of test plants oil on egg hatchability shows that *T. occidentalis* gave the lowest (2.27%) significant (p<0.05) percentage of eggs hatched while the control had the highest (91.0%) percentage. *C. olitorius* at all concentrations significant inhibited (p<0.05) hatching of eggs more than S. nigrum and *L. esculentum*. 2mg (68.1%) and 4mg (68.1%) of *S. nigrum* however had similar percentage of hatchability with 6mg (61.8%) of *L. esculentum* (Table 3 and Fig 2).

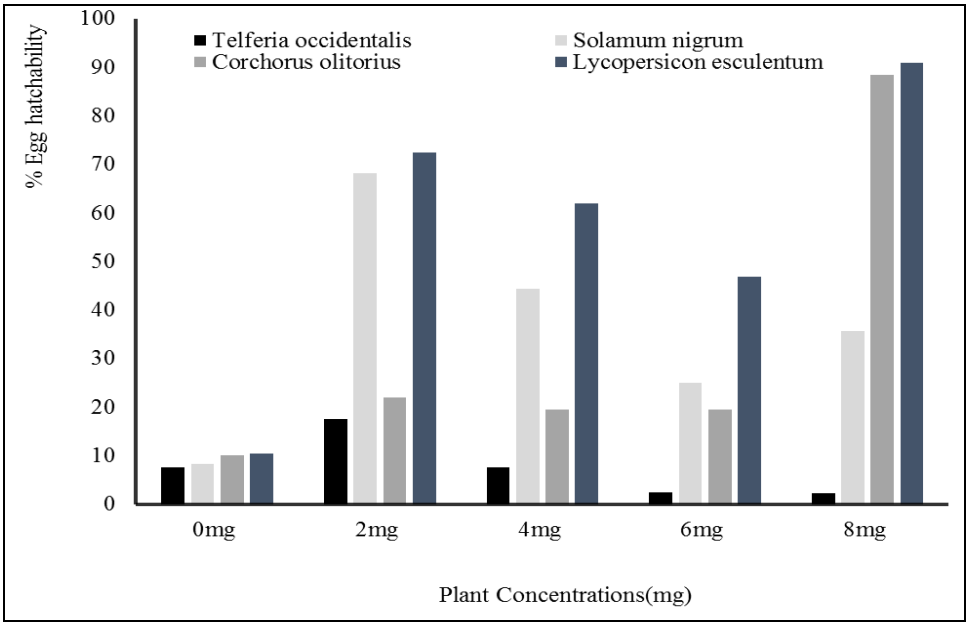

Fig 2: Effects of Plant Extract on egg hatchability of *D. maculatus*

Table 3: Effects of plant extracts on weight of smoked *Clarias gariepinus* infested by *Dermestes maculatus* during storage

Plant oil extracts	Mean initial weight (g)	Mean final weight (g)	Weight loss %
T. occidentalis	35.53 ± 0.52[a]	34.98 ± 0.56[c]	1.55[a]
S. nigrum	39.90 ± 0.49[a]	39.00 ± 1.02[d]	2.26[a]
C. olitorius	36.07 ± 4.03[a]	35.37 ± 5.63[c]	2.97[a]
L. esculentum	35.23 ± 2.43[a]	24.27 ± 1.11[a]	22.59[t]
Control	39.93 ± 3.46[a]	15.00 ± 1.69[a]	62.43[c]

Each value is a mean of the quadruplicate samples (±SE). Mean followed by the same superscript(s) in the same column are not significant different (p>0.05).

All treatment groups caused higher percentage of mortality of *D. maculatus* larvae then the control. *T. occidentalis* at 8mg and 6mg treatment levels had the highest significant (p<0.05) mortality of 90% and 80% respectively followed by 4mg of *T. occidentalis*; 4, 6 and 8mg S. nigrum and 8mg of *C. olitorus* which had comparable effects and higher % mortality than all other treatments groups and control. 2mg of *T. occidentalis*, 2mg of *S. nigrum*; 2, 4 and 6mg of *C. olitorus* and 2, 6 and 8mg of *L. esculentum* all had comparable effect. Control group however had similar effect as 2% *L. esculentum*. The effect of plant extracts on percentage weight loss in treated fish muscles indicated that control (treated) fish muscles had the highest significant weight loss (74.52%) followed by *L. esculentum* treatmet group (58.67%) while *S. nigrum* (6.80%), *C. olitorus* (10.66%) and *T. accidentalis* (4.24%) were not significantly different (p>0.05) and had similar least effect on weight loss (Fig. 3 and Table 4).

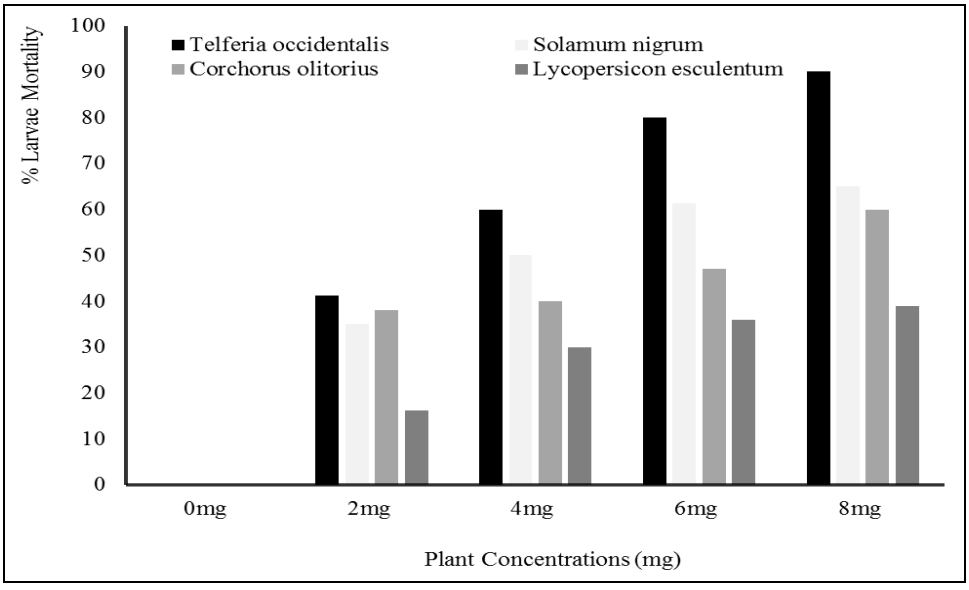

Fig 3: Effects of Plant extracts on Larval Mortality of *D. maculatus*

Table 4: Effects of Plant extracts on weight of *Clarias gariepinus* infected by *D. maculatus* during storage

Plant Treatment (oil)	Initial weight (g)	Final weight (g)	Weight loss	% weight loss
T. esculentum	98.50	41.00	57.50	58.67
S. nigrum	100.0	93.23	6.80	6.80
C. oliterus	98.50	88.05	10.5	10.66
T. occidentalis	105.5	101.05	4.45	4.24
Control	98.10	25.00	73.1	74.52

4. Discussion

The oil extracts from these plant materials were very potent in controlling the various stages of *D. maculatus* in smoked, *C. gariepinus*. This is similar to the report by [7] of the effect of application of oil extracts from the same plant materials on cowpea bruchid, *Callosobruchus*. Oil extract from *T. occidentalis, S. nigrum and C. olitorius* possess high potency in killing the adults, pupae, larvae and eggs of *D. maculatus* in smoked *C. gariepinus* during storage. This study revealed that oil extract of *L. esculentum* had the least significant difference (p>0.05) when compared with the oil extract of other plants used in this study and the control (untreated). Plants have phytochemicals which act as chemical defense against other organisms (such as insect) in the environment[8]. It is therefore possible that the strong pungent odour produced by these plants prevented D. maculatus from normal feeding thus resulting in starvation and subsequent death[9] and impairing the development of the immature stage of the insect pests. [6, 7, 8, 9] reported similar scenario when he used the extract from *Piper guineense, Monodora myristica, Aframomum melegueta, Tithona diversifolia and Nicotiana tobaccum* as surface protectants against the different stages (adults stage to the eggs) of fish beetle (*Dermestes maculatus*) (De geer) [9]. reported that oil extracts from *P. guineense, M. myristica and A. melegueta* showed significant difference (p<0.05) and was effective in the killing of all the adults, pupae and eggs of *D. maculatus* while the effect of oil extracts from *N. tobaccum* and *T. diversifolia* indicated no significant difference (p>0.05) from the untreated (control) smoked fish infected with the storage insect pest. Similar case of weight loss in both the treated and untreated fish muscles (which are used as indexes for calculating the rate of fish damage during storage) was also observed in the present study, and the percentage weight loss in fish during storage was significantly reduced (p<0.05) in fishes treated with oil extract from *S. nigrum* (6.80%), *C. olitorus* (10.66%) *and T. occidentalis* (4.24%) than *L. esculentum* (58.67%) and Control (74.52%) [8, 9]. This study reveals that certain plant oil extracts are effective in the prevention of *Dermestes maculatus* infestation of stored dried fish. *Telferia occidentalis* oil extract was highly active in killing the adult *D. maculatus* on the dried smoked fish. This was closely followed by oil extracts from *Solanum nigrum* at a treatment quantity but in the case of oil extract from the *Solanum nigrum* group. *Corchorus olitorius* oil extract at 8mg was significantly higher (p<0.05) than mortality obtained from treatment groups, the untreated control had the least mortality. *Lycopersicon esculentum* indicated the least mean value for all the treatment values for the mortality of *D. maculatus* but showed the highest mean value at 4mg for the formation of pupae in both the treated and untreated fish when compared to the other concentration levels and control respectively, while plant oil extracts from *T. occidentalis, S. nigrum, C. olitorius* indicated slight levels of concentrations. The mean larvae mortality of *D. maculatus* showed significance for *T. occidentalis, S. nigrum, C. olitorius* at 8mg concentration treatment level when compared to other concentration levels and control. On the egg hatchability rate of *D. maculatus,* groups treated with *T. occidentalis, S. nigrum* and *C. olitorius* oil extracts showed a significant decrease in the number of eggs hatched when compared to the control (untreated). This present study indicates that oil extracts from *T. occidentalis, S. nigrum* and *C. olitorius* are very effective in killing the adults, pupae, larvae and also deterring the egg hatchability of *D. maculatus* in smoked *C. gariepinus* during storage. This is in agreement with [10, 11] who reported that crude oil extract of the *T. occidentalis* (fluted pumpkin) at different concentrations was effective against *D. maculatus* while the oil of *Solanum nigrum* (garden egg) leaf caused a high mortality and repellency to the larvae of Dermestid [12, 3 13] reported that *C. olitorius* oil at dosages of 0.125ml/25g fish and 0.150ml/25g were effective in the control of larvae and adult stages of *D. maculatus* in dried *C. gariepinus.* This research has also shown that though the oil extract from *L. esculentum* had the least significant difference (p>0.05) in controlling *D. maculatus* during storage but effectively control in the development of the eggs of *D. maculatus* on the smoked *C. gariepinus* during storage. This is because some of these plants contain some insecticidal properties which impair the physical development of the various stages of insect pests [14, 5]. The result gotten from this study showed that the oil extracts of *T. occidentalis, S. nigrum* and *C. olitorius* were highly effective in controlling various stages of *D. maculatus* in smoked *C. gariepinus* during storage. Insect mortality in fish treated with the oil extracts of these plants materials was 100% effective compared with 16.7% in the untreated (control) smoked *C. gariepinus.* The effect of oil extracts from *Lycopersicon esculentum* (tomato leaf) on *D. maculatus* were not significant (p>0.05) from the control (untreated) smoked fish (*C. gariepinus).* This study revealed that smoked *C. gariepinus* is susceptible to *D. maculatus* infestation and thus has to be protected well during storage by technique that are environmental friendly and safe if nutrient loss is to be avoided.

5. Conclusion

This study reveals that, oil extracts from *T. occidentalis, C. olitorius* and *S. nigrum* were high in killing all the adults and larvae and also deterring egg hatchability of *D. maculatus* in smoked fish, *Clarias gariepinus* during storage. Investigation has proven that some of these plants are known to contain some insecticidal activities which can impair the physiological development of the stages of insect pest. In this regard, most of the plant oils have been reported to have surface tension, which may cause a reduction in the level of oxygen supply to the insects and consequently lead to suffocation and death. The effectiveness of these plant oil extracts in controlling insects pest during storage is probably a reflection of their insecticidal activities. The result showed that *L. esculentum* is not a good protectant against fish treated and the untreated in controlling the pest beetle, *D. maculatus* on smoked catfish, *C. gariepinus.* Promoting the use of these

plants oil extract will inhibit excessive and unnecessary wastage or cause damage to the fish during storage.

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