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Full Length Research Paper

BIO-EFFICACY OF THE ESSENTIAL OIL OF *THYMUS VULGARIS* AGAINST *DERMESTES MACULATUS* DEGEER 1774 (COLEOPTERA: DERMESTIDAE) INFESTING SMOKE-DRIED *CLARIAS GARIEPINUS* BURCHELL 1822

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ABSTRACT

Increase in demand for an alternative to conventional pesticides, resulted in testing various plants materials against different storage pests. In this study, white thyme (*Thymus vulgaris* L) essential oil (TEO) obtained by steam distillation using Clevenger apparatus was evaluated against two developmental stages of *Dermestes maculatus* by residual contact method under laboratory conditions (26^0C $\pm 2^0$C and 75% ±3% RH). Four concentrations (2.5%, 5%, 7.5% and 10%) of TEO were separately applied on fifteen grams of *C. gariepinus*. Newly emerged adults and 3rd instar larvae of the pest were exposed to the treated samples for a period of five days. Results showed that the TEO had significant ($P< 0.05$) effect on both adult and larval mortalities which was concentrations and time of exposure dependent. It was recorded that 7.5% and 10% concentrations resulted in 40% and 50% larval mortality of *Dermestes maculatus*48 HAE. Meanwhile 70% and 90% mortalities were observed in the same concentrations at 72 and 96HAE, respectively. On the other hand, adults were more susceptible than the larvae whereby higher (80% and 90%) mortalities were recorded within 24HAEin 7.5% and 10% concentrations, and complete(100%) adult mortality was observed at 48 HAE, respectively at the same concentrations. The LC_{50} of TEO against adults *D. maculatus* at 24 hours is 5.71% and against larvae is 13.93% with LT_{50} of 9.63h at 10% concentration against adult and 43.87h against larvae at the same concentration. Therefore, TEO can be used as an alternative to synthetic pesticides in the control of *Dermestes maculatus* infestations.

Keywords: Bio-efficacy, *Clarias gariepinus*, *Dermestes maculatus*, Essential oil, *Thymus vulgaris*, infestation

INTRODUCTION

Fish is one of the cheap animal protein source which makes a significant contribution to nutrition by providing readily available dietary nutrients to a large number of the people worldwide (Mufutau, 2012).It is consist of poly-saturated fatty acids, essential amino acids notably lysine, methionine, isoleucine, vitamins A, B, D, E, K and respectable amount of minerals such as phosphorus, calcium, iron, iodine, fluorine and magnesium (Okunade, 2011; Abolagba *et al*., 2015).Fishes is a source of income generation, poverty alleviation, foreign exchange earnings and raw materials for the animal feed industry (Akinwumi *et al*.,2007). Nigeria has an estimated annual per capita fish consumption of 17.5 kg, and a projected fish demand of 3.61 million metric tonnes (FDF, 2018). However, fish is easily perishable, especially in tropical climates, therefore, various preservation methods such as freezing, smoke-drying, sun drying and heat treatment are adopted after harvest, in Nigeria and Africa. To extend the fish shelf-life, ensure all season availability and meet diversified consumers taste (Babarinde, 2018).

The most prominent fish preservation method practiced is smoke-drying. About 80% of the captured fishes are smoke-dried and the remaining 20% is either salted, sun-dried, fried, or frozen (Ajao *et al*., 2018).*Clarias gariepinus* Buchell is the most widely smoke-drying fish in Nigeria. It is highly nutritious, economically important, and easily cultured (FDF, 2018). Despite the preservation method adopted, smoke-dried *C. gariepinus* have been reported of being infested by *Dermestes maculatus* L during storage. Various synthetic pesticides have been used and proved effective in the control and management of *Dermestes maculatus* infestation. However, these chemicals were scientifically discovered harmful to human health, non-target organisms and the environment at large. This elucidates the interest of testing the insecticidal properties of plant materials (Indabo and zakari, 2020). Therefore, the aim of this study is to evaluate the efficacy of the essential oil of *Thymus vulgaris* against two developmental stages of *D. maculatus* infesting smoked *C. gariepinus*.

MATERIALS AND METHODS

Collection and Preparation of Plants Materials

Dried leaves of *Thymus vulgaris* were purchased from Katsina Central market. Samples were then washed under running water in the laboratory and air dried under shade for a period of five days. The leaves were then kept in a well labeled plastic container (Soe *et al*., 2020).

Extraction of the essential oil

The essential oil from *Thymus vulgaris* leaves was extracted by steam distillation method, using Clevenger apparatus with some modifications. Seventy-five grams (75 g) of the dried leaves were taken into 1000 mL round bottom flask, and 750 ml of tap water was added in. The flask was heated on heating mantle for a period of three hours. The extracted essential oil along with the water vapor were evaporated through the connection tube and passed through the condenser. Distillate was collected in a separation funnel and water was drained into a beaker leaving the oil layers in the funnel.

Excess water was removed from the oil by passing over anhydrous sodium sulfate. The essential oil was then kept in a dark sealed vial at 4^0C (Ileke, 2021).

Collection and Rearing of *Dermestes maculatus*

Naturally infested smoke-dried *C. gariepinus* obtained from dry fish vendors at Katsina central market, was used as the source of *D. maculatus* culture for this study. The infested sample was taken in a transparent plastic container covered with muslin cloth. To postgraduate laboratory of the department of biology, Umaru Musa Yar'adua University Katsina (UMYUK). The insects were sorted, identified, and confirmed by entomology technician of the department, using the observable morphological features with the aid of hand lens (Olagunju, 2014). Un-infested smoke-dried *C. gariepinus* and the experimental jars were disinfested in dry air oven at 60^0C for 60 minutes to kill all possible insect pests and their eggs that might be present. Twenty pairs of identified beetles were introduced into three different rearing containers, containing 100g of disinfested smoked dried fish sample and water-soaked cotton wool. To serve as source of food as well as meeting the water requirement for oviposition, respectively. The rearing containers were then covered with muslin cloth, to prevent escape or entrance of unwanted insects. The set up was then kept in an incubator at a temperature of 28^0C ± 2^0C and 65% ± 5% relative humidity (R.H) for a period of two weeks to allow oviposition and larval emergence. After larval emergence, the adults were removed, and the newly emerged larvae were used to conduct Larvicidal bioassay and further reared the remaining to adult which were used for Adulticidal bioassay (Amadi and Dimpka 2018).

Larvicidal bioassay

To test the toxicity of *T. vulgaris* essential oils (TEO) against larva of *D. maculatus*, residual contact method maintained by Soe*et al.* (2020) was adopted. Four stock solutions (2.5%, 5.0%,7.5% and10% concentrations) were prepared using acetone as a solvent. After which 5ml of each of the oil solutions were thoroughly sprayed on 15g of disinfested smoked fish samples using a sprayer, each placed in different labeled transparent plastics container. The treated samples were air-dried for 60 min to allow acetone evaporation. Same grams of fish sample, treated with acetone only served as a control. Ten, 3rd instar larvae of *D. maculatus* were released on each treated fish flesh in the containers and covered with perforated lid fitted with net. To allow aeration an prevent entrance of some insects from outside. All treatments and control were replicated three times and kept in an incubator at 28^0C ± 2^0C and 65% ± 5% R.H. Larval mortalities were recorded every 24 for 4days and mean percentage larval mortality was calculated

Adulticidal bioassay

To test the toxicity of TEO against adult *D. maculatus*, residual contact toxicity method (Soe*et al*., 2020) was maintained. Fifteen grams (15g) of smoke-dried *C. gariepinus* were thoroughly treated with 5ml of each concentration of the prepared oil solutions. Treated fish samples were then placed in labeled transparent plastic containers. A

control was also prepared by spraying 5ml of acetone only, on the same grams of smoked dried fish. Each of the treatments and control were replicate three times. The treated samples were air-dried for 60 min to allow solvent evaporation. Five pairs of newly emerged (0-24 hours old) D. *maculatus* were released into each container and covered with a lid perforated and pitted with net from inside, to allow aeration, prevent the insects escaping and or intrusion of other insects from outside. All treatments and control were replicated three times and kept in an incubator at 28^0C ± 2^0C and 65% ± 5% R.H. Mortalities were recorded every 24 hours for five days. The insects were judged dead when they failed to respond to pin probe and mortalities were express in percentages.

Data Analysis

Mortality data were subjected to one way Analysis of Variance (ANOVA) at *p*= 0.05.Probit analysis was used to determine median lethal concentration (LC_{50}) and lethal time (LT_{50}) of TEO against larvae and adult of *D. maculatus* using SPSS version 26.0

RESULTS

Larvicidal Activity of TOE against *D. maculatus*

Result of larvicidal activity of TEO against *D. maculatus* is presented in Figure 1. The result shows that larval mortality increased with increase in the EO concentration and exposure period. Lower (6.67% - 30.00%) larval mortality was recorded in *C. gariepinus* treated with 2.5% - 5.0% concentrations, 24 – 96 hours after exposure (HAE). However, the result revealed that 40% and 50% larval mortality were recorded in *C. gariepinus* treated with 7.5% and 10% concentrations of TEO within 48 HAE. Furthermore, with extending the exposure to 72 - 96 hours, higher (70 – 93.3%) larval mortality was observed in these concentrations (Figure 1).

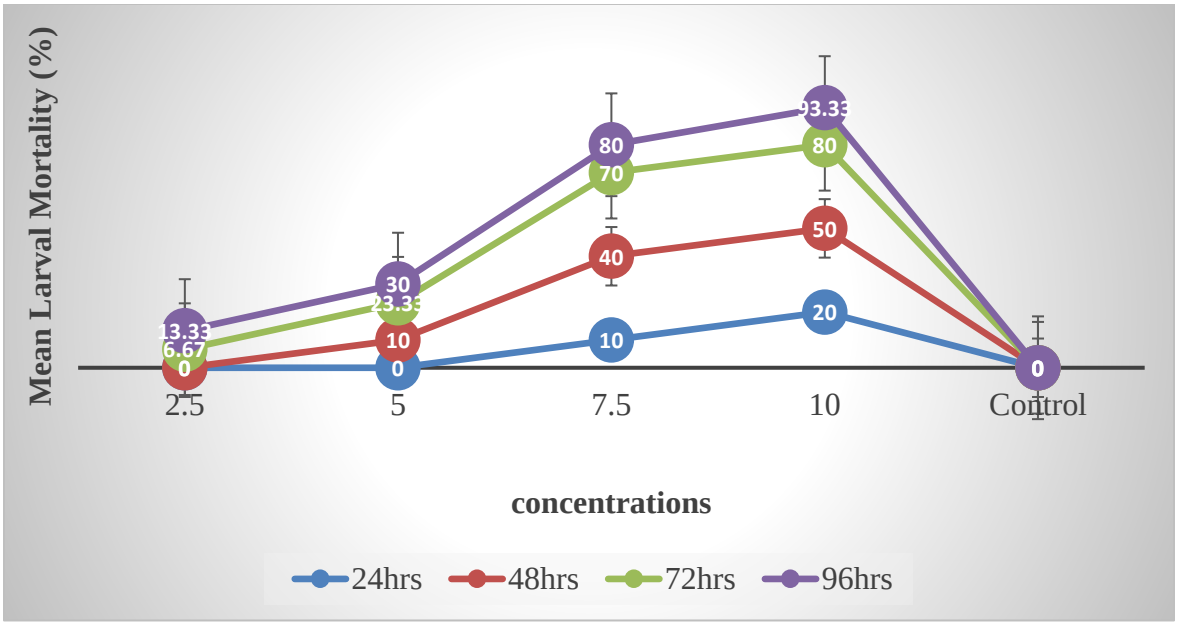


Figure 1: Larval Mortality of *D. maculatus* in Varying Concentrations of TEO Adulticidal Activity of TOE against *D. maculate.*

Figure 2, presented the result of adult mortality of *D. maculatus*exposed to *C. gariepinus* treated with varying concentrations of TEO. Result shows that higher (80.00 and 90.00%) adult mortality of *D. maculatus* were observed on *C. gariepinus* treated with 7.5% and 10% concentrations and lower (10.00% and 30.00%) mortality was recorded on fish samples treated with 2.5% and 5% concentrations of the EO, respectively at 24 HAE (Figure 2). Meanwhile, the mortality increased to 50.00% and 100% on *C. gariepinus* treated with 5.0% and 7.5% - 10% concentrations, respectively 48 HAE. Furthermore, as the exposure period extended to 72 and 96 hours, 70.00% and 80.33% adult mortality were recorded on 2.5% and 5.0% concentrations of TEO (Figure 2).

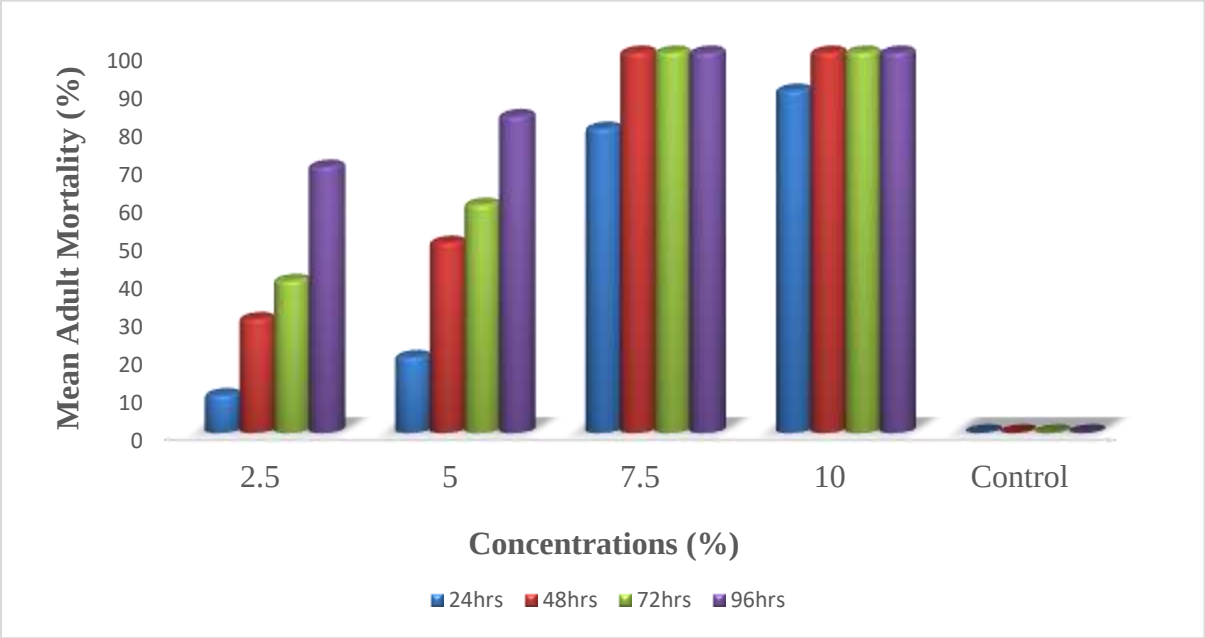


Figure 2: Adult Mortality of *D. maculatus* in Varying Concentrations of TEO

Median Lethal Concentrations (LC_{50}) and Median Lethal Time (LT_{50}) of TEO against Adults and Larvae of *D. maculatus*

The concentrations of TEO required to kill 50% of adult or larvae of *D. maculatus* is proportional to the exposure period; hence the LC_{50} of the EO against adult *D. maculatus* is lower (3.34%) at 72 HAE and higher (5.71%) at 24 HAE. Similarly, the LC_{50} of TEO against larvae was 6.30% and 13.93 at the same period of time, respectively. On the other hand, 2.5% concentration of TEO requires 73.43 hours to kill 50% adult of *D. maculatus*, while 5.0, 7.5 and 10% concentrations require 49.55, 13.55 and 9.63 hours to produce the same effect. Meanwhile the LT_{50} of TEO against larvae was 158.01, 131.24, 54.56 and 43.87 hours at 2.5, 5.0, 7.5 and 10% concentrations, respectively.

Table 1: Median Lethal Concentrations (LC_{50}) and Median Lethal Time (LT_{50}) of TEO against Adults and Larva of *Dermestes maculatus*

LC_{50} (%)		
Exposure period	Adult	Larva
24 hours	5.71	13.93
48 hours	3.74	9.66
72 hours	3.34	6.30

LT_{50} (hours)		
Concentrations (%)	Adult	Larvae
2.5	73.43	158.01
5.0	49.55	131.24
7.5	13.55	54.56
10	9.63	43.87

DISCUSSION

The essential oil of *Thymus vulgaris* was effective against the two developmental stages of *D. maculatus.* However, adults were more susceptible than the larvae and the effects were observed to be concentration and time of exposure dependent. The concentrations of TEO required to kill 50%of the pest (LC_{50}) were generally promising but higher against larvae than against the adults. Similarly, the time required to kill 50% of the pest (LT_{50}) is significantly higher in larvae than in adults at 2.5%, 5.0%, 7.5% and 10% concentrations of TEO. These results agree with the findings of Szczepanik *et al.* (2012) who reported that *Thymus vulgaris* essential oil, thymol and carvacrol resulted in 50.00%, 86.67% and 85.00% larval mortality of lesser mealworm (*Alphitobius diaperinus),* respectively at 1% concentrations. The mortality increased to 62.5, 91.67 and 97.5% with increase in concentrations to 2%. The result is also in agreement with the findings of Estekhdami *et al.* (2020) who reported that significant (56%) adult mortality of *Callosobruchus maculatus* was recorded in 0.25µl of thyme essential oil and further increase in concentration to 30µl resulted in 100% mortality of the pest. Further, Ogunduyile, (2015) reported that100% mortality *D. maculatus* larvae was recorded in fish treated with 0.256ml/g oils of Garlic, Coconut, Ginger, Jatropha, and Neem 96 hours after exposure. Akpotu *et al.,* (2017) reported larval mortality of *D. maculatus* as 3.33% - 81.11% and 2.22% - 91.11% at 0.027 mLg^{-1} and 0.081$mlg^{-1}$of the oils of *Citrullus vulgaris* and *Citrullus colocynthis*, respectively. Meanwhile, at 0.243mL^{g-1} both oils killed 100% of the insect larvae within 24hrsof exposure.

Contrary to this research, Ismail (2018) reported that the essential oil of *Thymus vulgaris* was not effective against two storage pests; lowest mortality of *Tribolium confusum* and *Tribolium castaneum* was

recorded in 5 ml of *Thymus vulgaris* oil/100g flour after 15 days. This might be as a result of differences in the bio-active compounds due to extraction method used, part of the plant used, region from which the plant was obtained. Because the insecticidal properties of green-pesticides depend on the bio-active compounds constituted therein that produce definite physiological actions on the insect body (Ukoroje and Otayor, 2020).

CONCLUSION

In view of the findings of this study, essential oil of *Thymus vulgaris* resulted in significant adult and larval mortality. Therefore, TEO could be used as an alternative to synthetic pesticides in the management of *D. maculatus* infesting smoke-dried *C. gariepinus.*

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