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Insecticidal Activities of total Crude Extracts of Citrus peels on *Dermestes maculatus (*DeGeer, 1774*)* pest of smoked fish

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Abstract

Insect pests often cause extensive damage to smoked fish which may lead to loss of value. The protection of smoked fish and agricultural products against insect infestation is of urgent need. Synthetic insecticides and fumigants are the main compounds used to preserve stored products which have detrimental effect on human and environment. The insecticidal activities of different Total Crude Extracts (TCE) of citrus (Citrus sinensis, C. limon, C. aurantifolia and C. reticulata) peels was carried out onlarvals of Dermestes maculatus to explore as alternative control measures to synthetic insecticides. The insect larvae were reared in a 1000ml bottle capped with a piece of 0.1cm mesh net to allow aeration and prevent escape of the larvae and entry of other insects. The fresh citrus peels were collected, dried under shade and ground into fine powder using mortal and pestle. The Total crude extracts were obtained at 10, 20, 30, 40, 50, 60, 70 and 80g/ml concentrations. The treatments were set in triplicates and left on a laboratory bench for 24 hours observation. The efficacy of the extracts increased with increase in concentration. The lemon (C. limon) TCE had the most significant mortality with 24h LC_{50} (55.26), which was closely followed by tangerine (C. reticulata) 24h LC_{50} (41.19) while the least effective extract was that of sweet orange 24h LC_{50} (40.36) and lime 24h LC_{50} (13.14). This result indicated that extracts formulations of lemon, tangerine, sweet orange and lime peels can effectively control fish pest. The use of total crude extract of citrus peels is considered effective as an agent in Integrated Pest Management (IPM) strategy with little or no health risk to humans and other living fauna in the environment.

Keywords: Insecticidal activities, Total Crude Extracts, Citrus peels, *Dermestes maculatus*

Activités insecticides des extraits bruts totaux d'écorces d'agrumes sur *Dermestes maculatus* (DeGeer, 1774) ravageur du poisson fumé

Résumé

Les insectes ravageurs causent souvent des dommages importants au poisson fumé, ce qui peut entraîner une perte de valeur. La protection du poisson fumé et des produits agricoles contre les infestations d'insectes est une nécessité urgente. Les insecticides et fumigants synthétiques sont les principaux composés utilisés pour conserver les produits stockés qui ont un effet néfaste sur l'homme et l'environnement. Les activités insecticides de différents Extraits Bruts Totaux (EBT) d'écorces d'agrumes (Citrus sinensis, C. limon, C. aurantifolia et C. reticulata) ont été menées sur des larves de Dermestes maculatus à explorer comme mesures de contrôle alternatives aux insecticides de synthèse. Les larves d'insectes ont été élevées dans une bouteille de 1000 ml bouchée avec un morceau de filet à mailles de 0,1

cm pour permettre l'aération et empêcher la fuite des larves et l'entrée d'autres insectes. Les écorces d'agrumes fraîches ont été recueillies, séchées à l'ombre et réduites en poudre fine à l'aide d'un mortier et d'un pilon. Les extraits bruts totaux ont été obtenus à des concentrations de 10, 20, 30, 40, 50, 60, 70 et 80 g/ml. Les traitements ont été fixés en triple et laissés sur une paillasse de laboratoire pendant 24 heures d'observation. L'efficacité des extraits a augmenté avec l'augmentation de la concentration. Le citron (C. limon) EBT avait la mortalité la plus importante avec 24h LC50 (55,26), qui était suivi de près par la mandarine (C. reticulata) 24h LC50 (41,19) tandis que l'extrait le moins efficace était celui d'orange douce 24h LC50 (40,36) et chaux CL50 24h (13.14). Ce résultat indique que les formulations d'extraits d'écorces de citron, de mandarine, d'orange douce et de citron vert peuvent contrôler efficacement les ravageurs des poissons. L'utilisation de l'extrait brut total d'écorces d'agrumes est considérée comme efficace en tant qu'agent dans la stratégie de lutte intégrée contre les ravageurs (ICR) avec peu ou pas de risque pour la santé des humains et des autres animaux vivants dans l'environnement.

Mots-clés : Activités insecticides, Extraits bruts totaux, Pelures d'agrumes, Dermestes maculatus

الأنشطة العرضية لمجموع المستخلصات الخام لقشور الحمضيات على آفة

الاسماك المدخنة

نبذة مختصرة

غالبًا ما تسبب الآفات الحشرية أضرارًا جسيمة للأسماك المدخنة مما قد يؤدي إلى فقد انقيمتها .حماية الأسماك المدخنة والمنتجات الزراعية من الإصابة بالحشرات هي حاجة ملحة. المبيدات الحشرية الاصطناعية والمبخرات هي المركبات الرئيسية المستخدمة للحفاظ على المنتجات المخزنة والتيلها تأثير ضار على الإنسان والبيئة .تم تنفيذ أنشطة المبيدات الحشرية لمختلف مستخلصات Ccrude (TCE) من الحمضيات (Citrus sinensis) و C. limon و C. aurantifolia و (C. reticulata على اليرقات من Dermestesmaculatus لاستكشافها كتدابير مكافحة بديلة للمبيدات الحشرية الاصطناعية . تمت تربية يرقات الحشرات في زجاجة 1000 ملمغطاة بقطعة شبكية 0.1 سمل لسماحب التهوية ومنع هروب اليرقات ودخول الحشرات الأخرى. تم جمع قشور الحمضيات الطازجة وتجفيفها تحت الظل وطحنها إلى مسحوق ناعم باستخدام مميتة ومدقة. تم الحصول على المستخلصات الخام الكلية بتركيزات 10 ، 20، 30 ، 40 ، 50 ، 60 ، 70 و 80 جم / مل. تم وضع العلاجات في ثلاث نسخ وتركت على مقعد معمل لمدة 24 ساعة للمراقبة .زادت فعالية المستخلصات معزيادة التركيز .كان لليمون TCE (C. limon) أكبر مع دلوفيات مع 24ساعة (55.26) LC50 ، والذي تبعه عن كثب اليوسفي (.C) والجير 24 ساعة .(13.14) LC50 أشارت هذه النتيجة إلى أن مستخلصات تركيبات الليمون واليوسفي والبرتقال الحلو وقشور الجير يمكن أن تكافح بشكل فعال آفات الأسماك. يعتبر استخدام المستخلص الخام الإجمالي لقشور الحمضيات فعالاً كعامل في استراتيجية المكافحة المتكاملة للآفات (IPM) مع وجود مخاطر صحية قليلة أو معدومة على البشر والحيوانات الحية الأخرى في البيئة.

المبيدات الحشرية، المستخلصات الخام الكلية، قشور الحمضيات؛:الكلمات المفتاحية

Dermestesmaculatus

Introduction

The frequent use of synthetic insecticide on stored products is not "friendly" to human health and the environment. This leads to biomagnifications, cancers and other serious ailments, and also environmental pollutions. Many natural products of plants origin are currently

being investigated to prevent these and other toxic effects on non-target organisms (Opender *et al.,* 2008; Abolagba *et al.,* 2011). The quest to reduce insecticide use on dried fish has made researchers employ alternative, eco-friendly, and cheaper insect-pest management measures involving plants and their products, like powders and extracts (Gonzalo, 2004; Mwanauta *et al.*, 2014). Insect pests often cause extensive damage to smoked fish, and this may amount to 5-10% in the temperate zone and 20-30% in the tropical zone (Haque *et al.,* 2000). In such a situation, the protection of smoked fish and agricultural products against insect infestation is urgent.

The citrus fruits extract are used in medicine such as Tylenol, the lime, lemon, orange and grape fruits extracts are mostly used for medicine production, the peel of citrus are also used as a facial cleanser (Jilani *et al.,* 2008). A peels of lemon and orange are commonly used as a means to moisten medical cannabis when stored with it. Lemons have the highest concentration of citrate of all citrus fruits, and daily consumption of lemonade has been shown to decrease the rate of stone formation (Slim, 2013). Citrus fruits have been observed to be used by Capuchin *(Cebus capucinus)* monkeys in Costa Rica as topical balms to protect against insects as well as an astringent and anti-fungal agent (Glob *et al.,* 2009).

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Material and Method

Study Area

This research was carried out in the Biological Science Department Laboratory, University of Abuja, Abuja, Nigeria.It lies on latitude 9.0765˚N of the equator and longitude 7.3986˚ E.

Preparation of Plant Powders

Fresh and ripe fruits of four citrus species (*Citrussinensis* - Orange, C. *aurantifolia* - Lime, *C. limon* - Lemon and C. *reticulate* - Tangerine) were purchased from Zuba fruit Market, Federal Capital Teritory, Abuja.They were peeled as shown in Plate 3 and air dried under room temperature (26-28˚C). Samples were pounded thoroughly using mortar and pestle and sieved through a 40holes/mm^2 mesh screen. Each plant powders was kept in a separate plastic container with a tightly fitted lid and placed in a cooled incubator at 24˚C for use in the experiment.

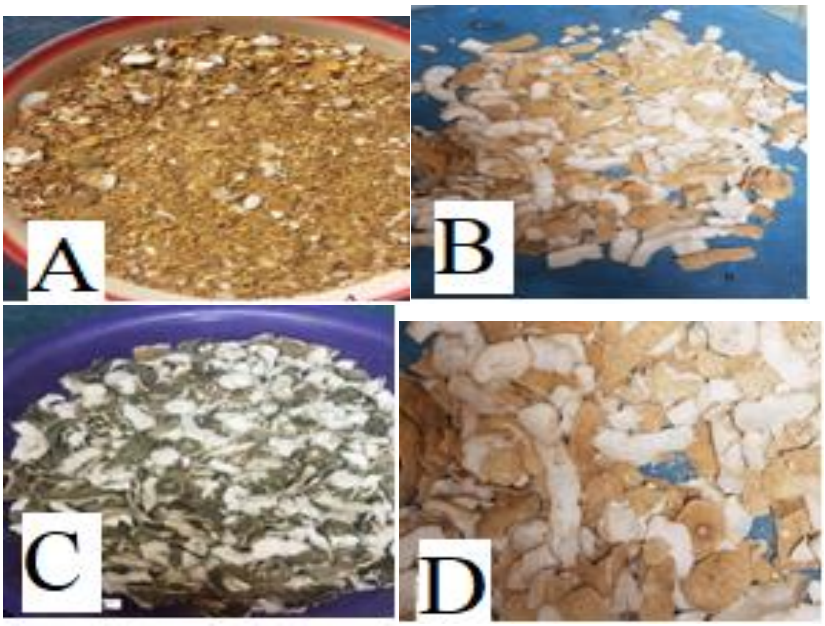

Plate 3: Citrus peels: (A) C. *sinensis* (Sweet Orange), (B) C. *aurantifolia* (Lime), (C) *C. limon* (Lemon) and (D) C. *reticulate* (Tangerine)

Plant Extracts Preparation

The method of Ohia *et al.* (2013) was employed and modified for preparation. The citrus (Orange, Lime, Lemon and Tangerine) peel powder(100g)was dissolved in 500ml of distilled water, then allowed to soak for 72 hours at room temperature but stirred regularly at every 12 hours. The resultant suspension was filtered into a 500 ml beaker using muslin cloth reinforced with Whatsman's No. 1 filter paper. The filtrates were evaporated in rotary evaporator to form a paste, and further transferred into sterile bottles and refrigerated until use.

Insect Culture and Fish Collection

The culture was collected from infested smoked *Clarias gariepinus* at Minna dried fish market, Niger State, Nigeria. The cultures were maintained separately in Kilner jars covered with muslin cloth under laboratory conditions at 30^{o}C and 75% (relative humidity. All bioassay jars were disinfected using the standard procedure by heat treatment in a Gallenkamp drying cabinets at 70^{o}Cfor 1 hour and allowed to cool at room temperature. The method of Adesina and Lajide (2012) was employed for new generation culture, where adults were removed from the stock culture, and the larvae were placed on fresh uninfected fish, then the parent adults were removed after 2-3 weeks of oviposition period. Moisture was supplied with pieces of soaked cotton wool to regulate temperature.

Effect of Plant Extract on Dermestes maculatus Larvae

The method of Adedire *et al.* (2011) was employed where ten 3rd instars larvae of *D. maculatus* were introduced into separate plastic jars containing 20g of disinfected dried fish muscles powder that had been thoroughly mixed with each of the plant extract at 0, 10, 20, 30, 40, 50, 60, 70 and 80g/ml concentrations. Tests were in triplicates for the eight treatmentsand were carried out at the ambient temperature32^{o}C and relative humidity of 60-70%. The jars were covered by mesh muslin cloth to prevent entry of other insects while allowing aeration. Larva mortality were recorded after 24hrs exposure for probit and lethal concentration analyses.

Data Analysis

The data from mortality of *D. maculates* against concentration of the methanolic extracts were computed, the means were computed and analysed with Chi-square, the probability and toxicity slope were computed, the values were exposed to Probit analysis using computer software Timkedward (1992) to calculation the LC_{50} at 95% confidence limit.

RESULT

Insecticidal properties of Total crude extracts of peels of orange species on D. maculatus larvae

The total crude extract effect of sweet orange peels on *D. maculatus* in a 24hrs static bioassay show increase in mortality rate due to concentration (Table 1), there were significant relationship ($P< 0.05$) expressed in Table 2 that give a linear curve shown in Fig 1 with Probit LC_{50} value of 40.36 g/ml.

The total crude extract effect of lemon peels on *D. maculatus* in a 24hrs static bioassay presented in Table 1, showing increase in mortality rate against concentration, with significant relationship ($P < 0.05$) expressed in Table 2 that give a linear curve shown in Fig 2 with Probit LC_{50} value of 55.26 g/ml.

The total crude water extract effect of lime peels on *D. maculatus* in a 24hrs static bioassay shows increase in mortality rate

against concentration, with significant relationship ($P < 0.05$) expressed in Table 2 that give a linear curve shown in Fig 3 with Probit LC_{50} value of 13.14 g/ml.

The total crude extract effect of tangerine peels on *D. maculatus* in a 24hrs static bioassay shows increase in mortality rate against concentration, with significant relationship ($P < 0.05$) that give a linear curve shown in Fig 4 with Probit LC_{50} value of 41.19 g/ml.

The total crude extract effect of lime peels showed less effect on the mortality of *D. maculatus*, while those of *C.reticulata* show 100% mortality on *D. maculatus* larvaeat 80mg/l those of *orange* and *lemon* showed 100% mortality on *D. maculatus* larvaeat 70mg/l showing that they are very effective as a control agent for the weevil.

Table 2: Probit analysis of Total crude extracts of Citrus peeles on D. maculatus larvae

Extract	Probit P-value	LC_{50}	Chi square P-value	Probit graph type
Citrus sinensis (sweet orange)	0.000	40.36	0.29	Linear
Citrus limon (lemon)	0.000	55.26	0.81	Linear
Citrus aurantifolia (lime)	0.013	13.14	0.96	Linear
Citrus reticulata (tangerine)	0.000	41.19	0.49	Linear

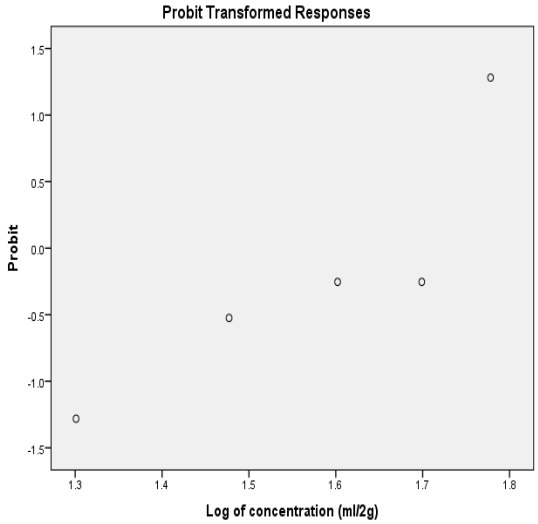

Figure 1: Probit curve of effect of *C. sinensis* (sweet orange peels) on *D. maculatus*

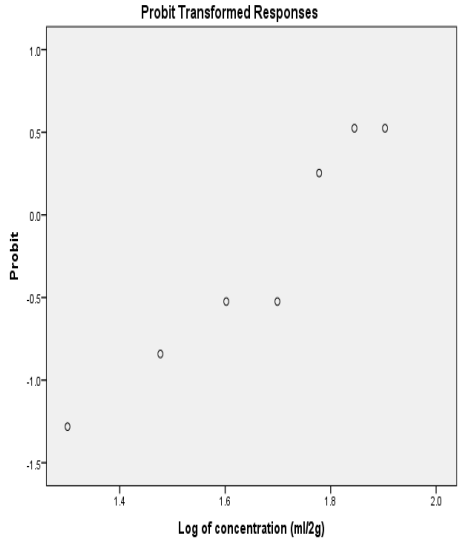

Fig 2: Probit curve of effect of *C. aurantifolia*(lime) on *D. maculatus* in a 24hrs static bioassay: total crude extract.

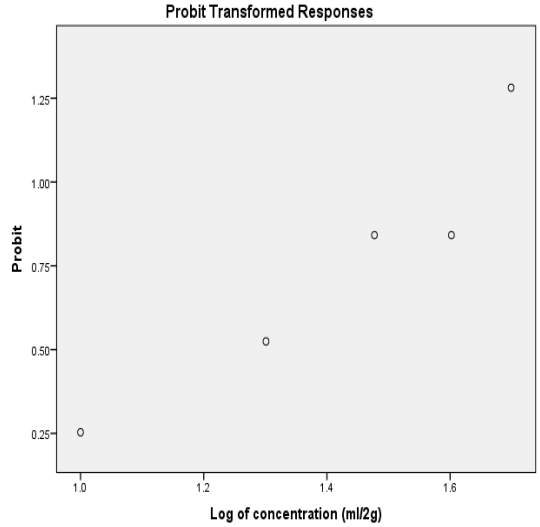

Fig 3: Probit curve of the Effect of *C. limon*(lemon) peels on *D. maculatus* in a 24 hrs static bioassay: total crude extract.

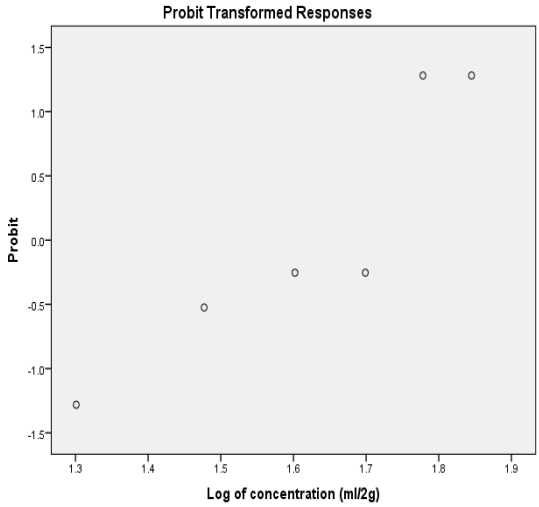

Fig 4: Probit curve of the Effect of *citrus reticulate* tangerine on *D. maculatus* total crude extract.

Discussion

The citrus peels extracts was found to have insecticidal effects against *D. maculatus* larvae. The insecticidal properties of the citrus peel extracts were influenced by the dosage and citrus species peels extracts applied. Lime peel total crude extract showed the highest efficient mortality trends at 24hours after application on the larvae. The peel extract with highest efficacy is lime on *D. maculatus* larvae with LC_{50} 13.14 g/ml.

Baidoo and Mochiah (2016) have shown that the major active ingredients of botanical extracts exert their bioactive against insects pest. The insecticidal effect of plant extracts may act as fumigant, repellent, stomach poison and physical barrier (block the spiracle and impair respiration). The extracts might reduce insect movement and also cause death through occlusion of spiracle thereby preventing respiration via trachea (Abdullah *et al*., 2017).

In this study, the larvae were rapidly killed by the extracts. Lale (2015) reported that plant extracts are highly lipophilic and could penetrate the cuticle of insects. The result obtained in the study is in line with (Aku *et al*.,2018) who reported that extract of *Anonna senegalensis* was more effective than the powder in the control of *D. maculatus.* Similarly. Odeyemi*et al*. (2019) observed that cases of high mortality occur in larvae partly because of their inability to detoxify plant toxins when feeding actively, especially at the 1^{st}-4^{th} instar larval stage. According to the authors, larvae are voracious eaters because of growth requirements, in contrast to the adult insects, which tend to have a reduced feeding habit.

Sabra and Mehana (2015) reported mortality as a major mechanism by which plant products control insect damage to stored fish. This view has been largely supported by the significant pesticidal effect of the extracts of the four plant materials investigated in this study. The rating of the extracts as promising pesticide in this study is partly in

agreement with (Egwunyenga *et al*., 1998) who also attributed the mortality of *D. maculatus* and *N. rufipes* from mixed fish tool factory to gustory sensations.

The use of the studied plant materials could be desirable in protecting smoked fish in the tropics, especially as organoleptic assessment has shown that treated fish do not exhibit adverse evidence of taint, smell or change in taste, texture or flavor (Akinwumi*et al.*, 2006). Equally the effect of the extracts may be due to the solubility potential of the different solvents as observed by Nwaehujor and Olatunji (2011).

Conclusion

The present laboratory investigation of extracts of peel of four *Citrus* sp. (*C. aurantifolia* (lime), *C. limon* (Lemon), *C. reticulata* (Tangerine) and *C. sinensis*(sweet orange) showed insecticidal property against *D. maculatus* larva. However, Total Crude extract from lime was found to have more insecticidal effect on D. *maculatus* larvae with 24h LC_{50} of 13.14.The 24h LC_{50} of tangerine was 41.19, while the least effective extracts were lemon with 24h LC_{50} of 55.26 and sweet orange with 24h LC_{50} of 40.36. This result indicated that extracts formulations of lemon, tangerine, sweet orange and lime peels can effectively control insect pest offish.

The effectiveness of the extracts increase with concentration. The use of citrus peel extracts needs to be encouraged for use at household level. The citrus peel extracts used in this study have shown that they can be developed as biopesticide for controlling stored product pest, particularly fish pest. The study is useful in developing plant extracts to reduce the use of synthetic chemicals in controlling *D. maculates* in smoked fish. Further work need to be done to reveal the bioactive ingredients of the peel extracts. The use of total crude extract of citrus peels is considered effective as an agent in Integrated Pest Management (IPM) strategy with little or no health risk to humans and other living fauna in the environment.

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