



Chemical Profile, Antifeedant, Insecticidal Activities, and Some Biochemical Properties of Two Essential Oils, Cyperus and Jojoba, Against the Rice Weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae)

Noura A. Hassan¹, Trandil F. Wahba²

1.Pesticide Chemistry & Technology Department, Faculty of Agriculture, Alexandria University, Egypt.

2.Insecticide Bioassay Department, Central Agricultural Pesticides Lab. (CAPL), Agriculture Research Center (ARC), Alexandria, Egypt.



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ABSTRACT: This study aimed to evaluate the antifeedant and insecticidal activities of two essential oils, Jojoba and Cyperus, against the adults of the rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and their impact on the activities of α -amylase and lipase enzymes, in addition to characterizing their chemical profile using GC-MS. The GC-MS analysis revealed that the major components found in Cyperus oil are alpha-linoleic acid (17.67%), linalool formate (12.30%), α - Ocimene (11.6%), and oleic acid (8.08%). whereas the major components found among 21 components in Jojoba oil are citronellol (17.95%), lemonol (9.88%), and (S)-citronellol acetate (7.40%). The insecticidal activity of Jojoba oil was significantly higher than that of Cyperus oil, with LC₅₀ values of 485.0 and 1700.73 mg/g for Jojoba and Cyperus oil, respectively. The results of antifeedant activity generally showed that the antifeedant activity of Cyperus oil was higher than that of Jojoba oil, and these results clearly appeared in the activities of both tested enzymes, α -amylase, and lipase, in treated insects with LC_{50s} of both oils, as the activities of them inhibited significantly compared to control, and this inhibition was more significant in the case of Cyperus oil.

Keywords: *Jojoba oil, Cyperus oil, antifeedant, Sitophilus oryzae*

INTRODUCTION

Many agricultural products, especially dried, stored, and robust products, are preferable for various stored product insects; also, many agricultural products that are value-added foods and nonfood derivatives are attacked by these insects (Phillips and Throne, 2010). Over one billion US dollars are lost due to the infestation of these insects per year worldwide (Boyer, Zhang, and Lempérière 2012). The nutritious value of infested stored grains decreased (Rajendran and Sriranjini, 2008) and became more vulnerable to mold infection, which causes mycotoxins production, the main dangerous issue in the production of livestock, in addition to the decrease in quantity, quality, and economic value as a result of the increasing activities of these insects (Yasothai, 2019).

Several techniques are developed by the approaches of Integrated Pest Management (IPM) to control grain infestation; nevertheless, the use of synthetic insecticides are employed by quality control (Kiran and Prakash, 2015), such as malathion, phosphine, pyrethroids, and chlorpyrifos, which were the most common all over the world. Although many undesirable effects result from the repeated application of these chemicals, such as the development of resistance

in insect pests towards these chemicals (Nayak *et al.*, 2014), the bad effects also extend to the environment and non-target animals (Isman 2006).

One of the alternatives for stored product pest control is plant extracts, which contain different essential oils that are volatile secondary metabolites that cause disruption in many functions of the insect body, including biochemical, behavioral, metabolic, and physiological processes. Also, these metabolites have been proven to cause inhalation, contact, and ingestion toxicity, antifeedant activity, the ability to cause an interruption in insect development, disruption in adult fertility and emergence, and disorder of oviposition in addition to repellent action (Tripathi *et al.*, 2001).

Recently, one of the most attractive types of essential oils has been jojoba oil, which is extracted from the jojoba plant, *Simmondsia chinensis* (L.) which is a monotypic species indigenous to North American deserts (McKeon, 2016). And cultivated in many countries around the world. (Khairi, 2019). Different studies concluded that jojoba oil has significant medicinal effects (Singh *et al.*, 2021). Additionally, it is reported to be toxic for the adults of the rice weevil, *S. oryzae* (L.) (Azab, 2018).

One of the medicinal plants that is native to India is *Cyperus rotundus* Linn., which has a large number of medicinal and pharmacological uses (N. Singh *et al.*, 2012). Additionally, it is reported to have insecticidal activity against some stored grain insects (Janaki *et al.*, 2018; Liu *et al.*, 2016).

The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), is one of the major grain pests that attack cereal kernels, as the results both quality and quantity are decreased during grain storage (Hong *et al.*, 2018).

This work aims to evaluate the chemical profiles of two EO_s, Cyperus and Jojoba, then investigate their antifeedant and adulticidal activities against the adults of *Sitophilus oryzae* and their effect on the activity of α -amylase and lipase enzymes in treated insects.

2. MATERIALS AND METHODS

2.1. Insect

The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), had been kept in the environmental toxicology lab for a period of more than ten years at the Faculty of Agriculture, Alexandria University. The insects were reared on sterilized whole wheat at $26 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH in complete darkness. The unsexed adult insects are used for the toxicity study 2-3 weeks post-emergence. All experiments were carried out under the same conditions.

2.2. Essential oils

Two essential oils, Cyperus and Jojoba, were purchased from Cleopatera essential oils Company, El-Mariotia Harm, Giza Road, Giza, Egypt.

2.3. GC-MS analysis of tested essential oils

The chemical composition of the tested essential oils Cyperus and Jojoba was analyzed using a GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m \times 0.25 mm film thickness). The initial temperature of the column oven was set at 50°C and increased gradually at a rate of $5^\circ\text{C}/\text{min}$ to reach 250°C , maintained for 2 min., then increased to (300°C) as the final temperature and maintained for 2 min. The temperatures of the injector and MS transfer line were kept at 270°C and 260°C , respectively. Helium was used at a constant flow rate of 1 ml/min as a carrier gas. The solvent delay was 4 min, and 1 μL of diluted samples were injected automatically through an Autosampler AS1300 coupled with GC in split mode. Electron ionization mass spectra were collected at an ionization voltage of 70 eV over a range of m/z 50-60 in the full-scan mode. The ion source temperature was set at 200°C . The chemical components of both essential oils were identified based on their retention time, and the mass spectra was compared with those of the Wiley 09 and

NIST 14 mass spectral databases. The percentage of components was calculated using the GC peak area. Their pH was measured using a pH meter (Crison™ pH-metro PH 25+).

2.4. Antifeedant bioassay

The antifeedant bioassay was conducted individually for each oil using the flour disc bioassay, described by (Koul, 2004) with slight modifications. Different concentrations of each oil were prepared in a total volume of 5ml of acetone, in addition to the control treatment (acetone alone). After that, all concentrations were stirred well with a suspension of wheat flour in water (2.5 g in 5 ml) to give the final concentrations (2000, 1000, 500, 250, 100 and 50 ppm). Aliquots of 200 μl of each concentration were pipetted into clean dishes to obtain small discs 1cm in diameter. The discs were left overnight to dry at $30 \pm 1^\circ\text{C}$ and $75 \pm 5\%$ R.H. for 12 h. The weight of the treated flour discs ranged from 0.04 to 0.05 mg. About 8-10 dried discs of each concentration were weighed and placed in a glass vial (5 cm in diameter by 10 cm in high). Insects were starved for 24h before the assays. Twenty unsexed adults (2-3-weeks-old) were weighed as a group and placed in each vial with five replicates for each concentration and control. Both discs and the live insects were reweighed after 7 days. Different antifeedant parameters were determined as follows:

Feeding Deterrence Index (FDI) (%) = $[(C - T)/C] - 100$, where C is the weight of the diet consumed in the control, and T is the weight of the diet consumed in the treated groups.

Relative growth rate (RGR) was calculated as G/I , where (G) is the change in insect weight, and (I) initial insect weight.

Relative consumption rate (RCR) as C/I where (C) is the change in diet weight, and (I) initial insect weight.

Efficiency of conversion of ingested food (ECI) as $100 * G/C$ where (G) is the weight gain of insect, and (C) weight of food consumed.

2.5. Toxicity tests

Different concentrations of each essential oil were prepared in acetone, and 1 ml of each concentration was applied to 20 g of wheat in a 0.25-liter glass jar. The jars were stirred continuously for 3 minutes to ensure the complete coverage of wheat grain with oil, and then the treated grains were left for 30 min to allow the complete evaporation of the solvent. Twenty adults were separately added to each jar, and the jars were covered with plastic caps. In addition, it was kept in the incubator under constant conditions ($26 \pm 2^\circ\text{C}$ and 70-80% RH). Mortality percentages were recorded after 7 days of exposure. LC_{50} values and their confidence

limits were calculated according to Finney (1971) using Ld-p Line® (software program).

2.6. Determination of α -amylase and lipase activities in treated insects

2.6.1. α -Amylase activity

Determination of α -amylase was conducted using α -amylase enzyme assay kit (BioSystems S.A. Costa Brava 30, Barcelona, Spain), which is based on a method in which α -Amylase catalyzes the hydrolysis of 2-chloro-4-nitrophenyl-maltotriose (CNP-G3) to 2-chloro-4-nitrophenol (CNP). The rate of 2-chloro-4-nitrophenol formation was measured at 405 nm according to Winn-Deen *et al.* (1988).

2.6.2. Lipase activity

Lipase activity was determined using a diagnostic kit, Lipase Kit S (Egyptian Co. for Biotechnology (Spectrum Diagnostics). 1, 2-o-dilauryl-rac-glycero-3-glutaric acid-(6'-methylresorufin) (DGGR) was used as a substrate. Absorbance was measured at 580 nm and 37°C using an automated analyzer, which is directly related to methyl resorufin production and lipase activity.

2.7. Data analysis

Data from the developmental study were recorded and expressed as means (S.E.). The analysis of variance (ANOVA) test was used to compare the

significance of mean differences between treatments and control at the 0.05% probability level with individual pairwise comparisons made using Tukey's HSD test through Co-Stat software.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of EOs

The chemical components of both essential oils, Cyperus and Jojoba, are listed in Table 1 as found by the GC-MS analysis. The chemical profiles of both EOs revealed the presence of 25 compounds in Jojoba oil and 21 compounds in Cyperus oil. The major components found in Cyperus oil are alpha-linoleic acid (17.67%), linalool formate (12.30%), α Ocimene (11.6%), and oleic acid (8.08%), whereas the major compounds found among the 21 components in Jojoba oil are citronellol (17.95%), lemonol (9.88%) and (S)-citronellol acetate (7.40%). The major constituents that formed both Cyperus and Jojoba essential oils were similar to previous reports. Awad *et al.* (2022) found that collated data from Egyptian farmers has a high percentage value of linoleic acid. Also, Hani *et al.* (2014) found alpha-linoleic acid to be the major component in Jordanian Jojoba oil. Araiza-Lizarde *et al.* (2017) considered linoleic C18: 2 (0.37 to 0.95%), and linolenic acid C18: 3 (0.87 to 2.50%) from total Jojoba oil fatty acids.

Table (1) GC-MS of Cyperus and Jojoba oil

| compounds | % Cyperus | % Jojoba oil | RT(min) |
|---|-----------|--------------|---------|
| Linalool | | 5.09 | 8.47 |
| Rose oxide | | 1.78 | 8.69 |
| Sextone | | 3.30 | 9.68 |
| α-Pinene | 3.51 | 0.68 | 11.01 |
| Citronellol | | 17.95 | 11.51 |
| Citronellyl formate | | 4.21 | 11.56 |
| Lemonol | | 9.88 | 12.05 |
| (S)-citronellol acetate | | 7.40 | 12.46 |
| Neryl formate | | 2.83 | 12.93 |
| (-)-α-Bourbonene | | 2.30 | 14.94 |
| oCymene | 1.01 | | 15.09 |
| Menthene | 3.79 | 4.86 | 15.20 |
| 5-Octen-2-one, 6-ethyl | 5.48 | | 15.41 |
| Caryophyllene | | 2.45 | 15.36 |
| Germacrene D | | 1.74 | 16.82 |
| Aromandendrene | | 1.52 | 17.14 |
| Calamenene | | 1.08 | 17.54 |
| δ-Cadinene | | 3.13 | 17.68 |
| Alloaromadendrene oxide-(2) | | 1.33 | 17.92 |
| Geraniol butyrate | | 1.86 | 18.25 |
| Linalool, formate | 12.30 | | 18.53 |
| 2-Phenylethyl tiglate | | 1.47 | 18.44 |
| epi-Eudesmol | | 6.70 | 19.43 |
| Agarospirol | | 1.06 | 19.67 |
| Benz[a]azulene-1,4-dione, 10-methoxy- | | 1.15 | 19.82 |
| Geranyl tiglate | | 1.95 | 20.82 |
| p-Menthan-1-ol | 0.98 | | 21.29 |
| α-Ocimene | 11.66 | | 24.14 |
| palmitic acid | | 2.06 | 25.51 |
| DLMalic acid | 1.13 | | 26.10 |
| Isocaryophyllene | 1.45 | | 29.65 |
| α-Himachalene | 1.01 | | 30.62 |
| α-Curcumene | 1.07 | | 31.53 |
| (+)-2-Carene | 1.35 | | 32.22 |
| Caryophyllene oxide | 0.85 | | 34.83 |
| nHexadecanoic acid | 3.24 | | 45.00 |
| alpha-Linoleic acid | 17.67 | | 48.73 |
| Oleic Acid | 8.08 | 1.34 | 48.78 |
| Octadecanoic acid | 1.81 | | 48.98 |
| 15-Hydroxypentadecanoic acid | 1.63 | | 50.68 |
| Z(13,14-Epoxy)tetradecan-1-yl acetate | 1.24 | | 51.56 |
| Monoelaidin | 2.55 | | 52.84 |
| Cyclopropaneoctanoic acid, 2[(2-pentylcyclopropyl)methyl], methyl ester | 8.06 | | 53.14 |

3.2. Toxicity bioassay

The insecticidal activity of both EOs was evaluated against the adults of *S. oryzae*, and the data was recorded after 7 days. Table 2 shows the LC_{50s} of both Eos. The results reported that Jojoba oil exhibited toxicity that was significantly higher than Cyperus oil; the LC_{50s} recorded for both EOs

were 485.0 and 1700.73 mg/kg for Jojoba and Cyperus oils, respectively. Many researchers found that Jojoba and Cyperus oils have an insecticidal effect. The Jojoba oil has insecticidal activity against *S. oryzae* on wheat with LC₅₀ of 3.12 mg/kg (Abdel-Razik and Mahmoud, 2017). The adulticidal activity of jojoba oil against *S. oryzae*

at $28 \pm 2^\circ\text{C}$ which increased with increasing the time of exposure, LC_{50} s were 0.171, 0.017 and 0.052 % after 7,10 and 14 days after exposure (Azab, 2018). The evaluated LC_{50} of Cyperus oil against the adults of *Oryzaephilus surinamensis*, *Trogoderma granarium*, and *Callosobruchus maculatus* were

0.51, 0.20 and 0.34 ($\mu\text{L}/\text{cm}^2$) (Janaki *et al.*, 2018). Also, the toxicity of Cyperus oil was reported against the adults of *Sitophilus oryzae* (LC_{50} s were 221, 341, 246, and 313 ppm) after 72 hours on four wheat cultivars (Misr1, Giza171, Gemmeiza12 and Sids14) (George *et al.*, 2023).

Table (2) LC_{50} s of tested EOs Cyperus and Jojoba.

| Essential oil | LC_{50} (mg/g) | Confidence limits Lower –upper | X2 | Slope \pm SD |
|---------------|-------------------------|-----------------------------------|------|------------------|
| Cyperus oil | 1700.73 | 930.02-3928.12 | 0.54 | 0.58 \pm 0.17 |
| Jojoba oil | 485.06 | 399.70-592.85 | 1.85 | 1.90 \pm 0.218 |

3.3. Antifeedant bioassay

Table 3 shows the Feeding Deterrence Index (FDI) and Relative Consumption Rate (RCR) for flour discs treated with different concentrations of Cyperus and Jojoba oil. In general, the results showed that the antifeedant activity of Cyperus oil was more effective than that of Jojoba oil. The FDI value for both EOs increased with the oil concentration increase, and the concentration of

2000 mg/g caused the most significant increase of FDI compared to the control in both EOs (94.52 for Cyperus oil and 83.50 for Jojoba oil). In contrast, the value of RCR for both EOs decreased with concentration increase, similarly, the concentration of 2000 mg/g caused the most significant depression in the value of RCR compared to the control in both tested EOs (0.26 g/g for Cyperus oil and 0.4 ± 0.1 g/g for Jojoba oil).

Table (3): Feeding Deterrence Index (FDI) and Relative consumption rate (RCR) for flour disks treated with different concentrations of Cyperus and Jojoba oils

| Conc. (mg/g) | Cyperus oil | | Jojoba oil | |
|--------------|--------------------------------|------------------|--------------------------------|------------------|
| | Feeding Deterrence Index (FDI) | RCR (g/g) | Feeding Deterrence Index (FDI) | RCR (g/g) |
| 0.0 | ± 0.00 d0.00 | 5.33 \pm 0.09a | 0.00 \pm 0.00d | 5.28 \pm 0.9a |
| 250 | 51.03 \pm 1.86c | 2.89 \pm 0.08b | 58.55 \pm 1.16c | 2.55 \pm 0.15b |
| 500 | 61.34 \pm 1.16b | 2.29 \pm 0.09c | 65.23 \pm 1.20bc | 2.09 \pm 0.09c |
| 1000 | 65.47 \pm 0.48b | 1.86 \pm 0.09d | 65.63 \pm 0.43b | 1.90 \pm 0.81c |
| 2000 | 94.52 \pm 0.46a | 0.26 \pm 0.06e | 83.50 \pm 0.86a | 0.4 \pm 0.1d |

The relative growth rate (RGR) and efficiency of conversion of ingested food (ECI) for *S. oryzae* treated with different concentrations of Cyperus and Jojoba oil are represented in Table 4. Both ECI and RGR values decreased significantly with concentration increase compared to the control. For Cyperus oil, the concentration of 2000 mg/g

caused the most significant decrease in both parameters compared to the control (ECI= 4.99 % and RGR=0.01g/g). while for jojoba oil, the concentration of 500 mg/g caused the most significant decrease in both parameters compared to the control (ECI= 5.09 % and RGR = 0.11g/g).

Table (4): Relative growth rate (RGR) and Efficiency of conversion of ingested food (ECI) for *S. oryzae* treated with different concentrations of Cyperus and Jojoba oils.

| Conc. (mg/g) | Cyperus oil | | Jojoba oil | |
|--------------|--------------------|------------------|-------------------|-------------------|
| | ECI (%) | RGR (g/g) | ECI (%) | RGR (g/g) |
| 0.0 | 15.63 \pm 0.53a | 0.82 \pm 0.01a | 13.77 \pm 1.42a | 0.72 \pm 0.06a |
| 250 | 12.43 \pm 0.31b | 0.34 \pm 0.01b | 6.34 \pm 0.51b | 0.15 \pm 0.00b |
| 500 | 11.86 \pm 0.68bc | 0.28 \pm 0.00b | 5.09 \pm 1.06bc | 0.11 \pm 0.02bc |
| 1000 | 9.86 \pm 0.81c | 0.18 \pm 0.02c | 3.18 \pm 0.35bc | 0.06 \pm 0.00bc |
| 2000 | 4.99 \pm 0.41d | 0.01 \pm 0.00d | 2.14 \pm 0.17c | 0.01 \pm 0.00c |

In concordance with our results, Brari and Thakur (2016) found that citronellol has antifeedant activity of 65.21% against *Sitophilus*

oryzae, 65.4% oviposition deterrence against *Callosobruchus analis*, and revealed 82% mortality of *C. analis* and 73% of *S. oryzae* adults

with dose of 10 µl/cm² after 10 days (Brari and Kumar, 2020). Citronellol showed antifeedant activities of 78.95% and 52.80% at concentrations of 30 µl/g against *Tribolium castaneum* and *S. oryzae*, respectively. Linalool is also one of *S. chinensis* EO components that has an antifeedant effect according to (Kostić *et al.*, 2008) who found that Linalool's antifeedant index was relatively high against the 2nd instar larvae of *Lymantria dispar* and decreased *Prunus cerasifolia* leaf mass damage. Therefore, citronellol and linalool might

be one suitable oil components that have biological control over many agricultural insects (Pajaro-Castro *et al.*, 2017; Kheloul *et al.*, 2020).

3.4. In-Vivo interaction of tested EOs with amylase and lipase enzymes.

Adults of *S. oryzae* were treated with LC₅₀ values of both EOs, then the α-amylase and lipase enzymes were extracted, and their activity was determined. The results are shown in Table 5.

Table (5) In-vivo inhibition of lipase and α-amylase enzymes extracted from *S. oryzae* treated with LC_{50s} of Cyperus and Jojoba oils.

| Essential oil | lipase IU/mg protin ±ES | α-amaylase IU/mg protin±ES |
|---------------|-------------------------|----------------------------|
| Control | 2.25±0.02a | 10.00±0.59a |
| Cyperus oil | 1.35±0.02c | 2.25±0.08c |
| Jojoba oil | 2.00±0.05b | 3.95±0.08b |

Both enzymes were significantly inhibited in both tested oils compared to the control, and the Cyperus oil caused a significant inhibition more than Jojoba oil in the activity of both enzymes. Mehrabadi *et al.* (2011) reported the inhibition effect of some plant extracts, *Peganum harmala*, *Punica granatum L.*, *Artemisia sieberi B. L.*, *Rheum officinale B.*, *Datura stramonium L.*, *Rhus coriaria L.*, and *Thymus vulgaris L.* on amylase activity of *Rhyzopertha dominica F.*, *Sitophilus granarius L.*, *Callosobruchus maculatus F.*, and *Trogoderma granarium E* (Mehrabadi *et al.*, 2011). Also, the EOs from Siam and vetiver leaves reduced the activity of the house fly lipase (Soyelu *et al.*, 2020). Also, the effect of menthol on the digestive enzyme's activity was studied and found to cause disruption in the enzyme's activity in treated adults of *Rhyzopertha dominica* compared to controls. A reduction in the specific activity of α-amylase, protease, lipase, and chitinase was obtained (Tine-Djebbar *et al.*, 2023).

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الملخص العربي

السّمات الكيميائيّة والنشاط الإبادى ومانع التغذية وبعض الصفات البيوكيميائية لاثنين من

الزيوت العطرية، زيت السعد وزيت الجوجوبا ضد حشرة سوسة الأرز

نورة عبد الفتاح حسن¹، ترانديل فايز وهبة².

1-قسم كيمياء وتكنولوجيا المبيدات - كلية الزراعة - جامعة الاسكندرية - مصر

2-قسم بحوث التقييم الحيوي - المعمل المركزي للمبيدات الزراعية - مركز البحوث الزراعية - مصر .

تستهدف الدراسة الحالية تقييم النشاط الإبادى وتأثير منع التغذية لاثنين من الزيوت العطرية وهما زيت السعد وزيت الجوجوبا ضد الحشرات البالغة لسوسة الأرز وكذلك تأثيرهم على نشاط كل من انزيم ألفا-أميليز والليباز بالإضافة الى دراسة التركيب الكيميائى لكلا الزيتين بإستخدام التخلييل كروماتوجرافى الغاز GC-MS والذى أظهر أن أغلب مكونات زيت السعد هي (8.08%) بينما المركبات الأكثر تواجدا فى زيت الجوجوبا كانت (S)-citronellol acetate (7.40%). أوضحت النتائج أن زيت الجوجوبا له تأثير إبادى أعلى من زيت السعد حيث تم تسجيل قيم LC50 لكلا الزيتين كالتالى 485.0 و 1700.73 مجم/جم لزيت الجوجوبا وزيت السعد على التوالى بينما نتائج دراسة تأثير منع التغذية أظهرت الفاعلية العالية لزيت السعد مقارنة بزيت الجوجوبا ووضحت هذه النتيجة عند تقدير نشاط انزيميى ألفا أميليز والليباز حيث إنخفض نشاط كلاهما مقارنة بمعاملة المقارنة وكان الإنخفاض معنوى أكثر فى حالة زيت السعد.