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The antifeedant properties of bio-oil from Cupressus sempervirens against rice weevil (*Sitophilus oryzae*) compared to that of Myrrh and Frankincense oils

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ABSTRACT

Bio-oils are a complex material that is produced through the condensation of vapors raised from the pyrolysis of plant biomass components and consist mainly of phenolic compounds, benzene derivatives, and organic acids. Few studies have been conducted on the antifeedant efficiency of bio-oils. This study aims to evaluate the chemical composition, toxicity, and antifeedant activity of bio-oil produced from the pruning residue of *Cupressus sempervirens* in addition to two types of essential oils, Myrrh and Frankincense oil, against *Sitophilus oryzae*. The GC/MS analysis of the essential oils showed Diethyl Phthalate (75.15%) in myrrh oil, alpha-Thujene (30.70%), and Octyl acetate (20.44%) in frankincense oil. The results clearly showed the high toxicity of essential oils, myrrh and frankincense, and the low toxicity of bio-oil against *S. oryzae* after 7 days of exposure time, while the while the combined use of myrrh oil and frankincense oil with bio-oil increased the toxicity against *S. oryzae* about 1.91 and 2.27-fold, compared to myrrh oil and frankincense oil alone. In all treatments, the antifeedant parameters revealed that myrrh oil had higher nutritional indices than Frankincense oil. *C. sempervirens* showed a significantly low antifeedant efficacy compared to both essential oils. Generally, the values of relative growth rate (RGR), relative consumption rate (RCR), and efficiency of conversion of ingested food, (ECI) significantly decreased, and, contrariwise, the FDI increased in all treatments compared to the control.
Keywords: Bio-oil, essential oil, antifeedant, Myrrh oil, Frankincense oil, *Sitophilus oryzae*.

INTRODUCTION

The presence of insects in stored grains always leads to significant losses in both quality and quantity of food; a decrease in nutritional value; and a loss of marketing value due to the contamination with insects' body waste. Also, the damage caused to the grains led to their being attractive to other poisonous insects and organisms (Licciardello, 2018). In addition to attacking the seed embryos and negatively affecting the germination of those seeds (Baier and Webster, 1992; Brari and Kumar, 2019). Generally, the order Coleoptera is one of the largest insect orders and includes different species of economically important insects that attack stored products (Shankar and Abrol, 2012). The damage of stored grains caused by insects is estimated to reach 40% in developing countries (Safavi and Mobki, 2016). So far, the management process of stored product insects is principally based on the application of synthetic insecticides, which mainly belong to the organophosphates, pyrethroids, and neonicotinoids groups, in addition to using phosphine as a fumigant (Agrafioti and Athanassiou, 2018; Tsaganou *et al*., 2021). Nevertheless, the extensive application of these chemicals increases significant worries about the negative impacts on human health due to the accumulation of its residues on grains, the development of insect resistance, and negative impacts on the environment. (Shawer *et al.,* 2022). So, it has become very necessary to search for eco-friendly alternative approaches to control these insects (Shawer, 2017; Shawer *et al.*, 2018). In this regard, the most promising alternatives are natural and non-persistent insecticides (Saroukolai *et al*., 2010; Ebadollahi *et al.,* 2021; Narayanankutty *et al*., 2021; Garrido-Miranda *et al.,* 2022). One of the most promising natural products plant essential oils (EOs), which have different effects on insects by acting as insecticidal compounds, oviposition, antifeedants, or repellents for stored product insects (Perera *et al.*, 2022). The extracts of several EOs that are derived from aromatic plants have been extensively investigated for their efficiency against different insect species and have shown promising results (Isman, 2000; Abdelgaleil *et al.,* 2015; Ju *et al*., 2019; Mehta and Kumar, 2020).

Bio-oil, tar, and biochar are organic materials that are produced through the eco-friendly pyrolysis process (Pisa *et al.*, 2021). Plant biomass components bio-oil is a complex material that is produced through the condensation of vapors raised from the pyrolysis of plant biomass components (Grewal *et al*., 2018) and consists of several compounds, which are mainly phenolic compounds, benzene derivatives, and organic acids (Mathew and Zakaria, 2015). Several studies have been conducted on the insecticidal, bactericidal, and fungicidal activities of bio-oil (Chalermsan and Peeran, 2009; Wititsiri, 2011; Ferreira *et al.,* 2013), its repellency effect on some insect species (Kiarie-makara *et al.,* 2010), and how it acts as a plant growth enhancer (Mu *et al.,* 2006). Numerous studies have discussed the insecticidal efficacy of bio-oil, but the effect of preventing the feeding of bio-oil on insects has been little explored. From this standpoint, this study aims to study the chemical composition of bio-oil produced from pruning residue of *C. sempervirens* (Mediterranean cypress) trees in addition to two types of EOs, Myrrh oil, and Frankincense oil, and then evaluate the insecticidal and antifeedant efficiency of bio-oil and both EOs against the rice weevil, *Sitophilus oryzae*.

MATERIAL AND METHODS

Insect culture:

A culture of *Sitophilus oryzae* was established depending on an original culture reared for several generations away from any insecticidal contamination in the laboratory of environmental toxicology at the Department of Pesticide Chemistry and Technology, Faculty of Agriculture, Alexandria University, Egypt. The jars were covered with muslin cloth and kept in a growth cabinet with a temperature of 30 ± 2ºC, a relative humidity of 70±5%, and a 12:12 light: dark photoperiod.

Essential oil analysis of myrrh and frankincense:

Myrrh oil and Frankincense oil were obtained from Cleopatra essential oils' company, El-Mariotia Harm – Giza Road, Giza, Egypt. The chemical composition analysis of myrrh and Frankincense essential oils was done using the MS engine 5989B in the EI mode in conjunction with the HP 5890 gas chromatography-mass spectrometry system. Samples that were injected were diluted in 1 ml of diethyl ether and 1 l of injection fluid. The GC was outfitted with a solid-phase Rtx-5MS, 30m x 0.25 mm, 0.25 m solid-phase 5%diphenyl-95%dimethyl polysiloxane capillary column. The GC conditions were 50 °C (2') to 250 °C for 10 minutes at a rate of 8° C/min, and 1 ml/min of helium flow made up the temperature program. Similar procedures were also used with a Thermo Finnegan GC-MS fitted with an Rtx-5MS (30m x 0.25mm, 0.25m) column. The ion source and the GC-MS interface line were kept at 200 °C or 250 °C. The electron emission was 100 A, with an electron energy of 70 eV. RES.

Bio-oil preparation and refinement from *C. sempervirens* wood:

The raw material for bio-oil was branches of pruning residue of *C. sempervirens* (Mediterranean cypress) trees grown in the forestry research sector of the Antoniadis botanical garden, Alexandria governorate, Egypt. The pruned branches were air-dried in the open field for about six months, and then the branches were stored at room temperature for about two years. The branches were debarked and sawn into suitable pieces.

A metal cuboid-shaped reactor was measured at 200, 130, and 50 mm in length, width, and height, respectively. An electrically heated furnace and a temperature controller were included. In the related test, 100 g of wood sample was heated in the reactor at a rate of around 5 °C/min from room temperature to the target temperature. The samples were heated to a pyrolyzing temperature of 400 °C. The required temperature was maintained for 1 hour. For the separation of pyroligneous acids, the pyrolyzed vapors were passed through a cooled condenser. The bio-oil was stored in a refrigerator after the pyrolysis experiment, and as the storage period extended, the crude bio-oil was gradually characterized into three different layers. The top layer was thin oil, the middle layer was high-quality liquid, and the bottom layer was viscose wood tar mixed with a variety of other materials. It was possible to separate the liquid into the intermediate fraction, which will be bio-oil.

Chemical component analysis bio-oil:

Bio-oil from *C. sempervirens* Thermo Scientific's GC-TSQ mass spectrometer and TG-5MS direct capillary column was used to analyze the chemical components of *C. sempervirens* bio-oil (30 m x 0.25 mm film thickness). The temperature of the column oven was initially maintained at 50°C, increased by 5°C/min to 250°C, held for 2 minutes, and then increased to the final temperature of 300°C by 30°C/min, held for 2 minutes. Helium was used as the carrier gas, with a constant flow rate of 1 ml/min, and the temperatures of the injector and MS transfer line were maintained at 270 and 260 °C, respectively. Using an Autosampler AS1300 connected to GC in the split, diluted samples of 1 l were automatically injected with a 4-minute solvent delay.

Toxicity of myrrh oil and frankincense oil:

A series of concentrations of tested essential oils of 100, 200, 500, 1000, and 2000 mg/kg were dissolved in (1 ml) acetone and applied to the 20 g of wheat in a 0.25L glass jar. The jars were vigorously stirred continuously for 1 min to ensure the most recent spread of oil on the wheat grain, and the solvent was allowed to evaporate for 15 minutes completely. Twenty adults were placed in each jar and covered with a cap. Also, it was kept in the incubator under constant conditions (27 ± 2 0C and 70-75% RH). Each concentration was replicated in four replicates. Mortality percentages were recorded after 7 days. LC_{50} values and their confidence limits were calculated according to Finney (Finney, 1971) using Ld-p Line®.

Toxicity of bio-oil:

The bio-oil was prepared in dilutions of 50,000, 100,000, 200,000, and 300,000mg/kg with acetone. 20 g of sterile wheat should be added to a 250 mL glass jar along with the dose rates. All of the jars containing sterilized wheat and bio-oil were physically shaken to ensure that the bio-oil coated the grain evenly. Bio-oil was allowed to evaporate for a full night at 50 degrees Celsius. Twenty adults were placed in jars on their own. The only control used was acetone. Each treatment was replicated in four replicates. The mortality rates were reported after 7 days of treatment.

Testing the combination of the tested oils and bio-oil:

The bio-oil at a dilution of 300000mg/kg was applied into jars containing 20g of wheat. All treatments were allowed to evaporate overnight at 50 ^{o}C as a pretreatment. Then, the LC_{50} values of the oils were added as previously. Mortality percentages were recorded after 7 days of treatment.

Antifeedant bioassay:

Evaluation of the antifeedant activity of the obtained oils was performed using a flour disc bioassay described by (Koul, 2004) with minor modifications. 100 µl oil was dissolved in 2 ml of acetone to obtain different solution concentrations of 0.005, 0.01, 0.016, 0.032, 100, and 0.125 mg/g, as well as control, which were prepared using acetone alone. Following that, the final solutions (5ml) of various concentrations were thoroughly mixed with the wheat flour suspension in water (2.5 g in 5 ml). Aliquots of 200 µl of different concentration suspensions were transferred into clean dishes to form small discs 1 cm in diameter. The discs were dried overnight before being equilibrated at 30 ±1°C and 75-±5 % R.H. for 12 h. The weight of the four discs ranged between 0.04 and 0.05 g. approximately 8-10 dried discs were weighed and placed in a glass vial for each concentration (5 cm in diameter by 10 cm high). Twenty unsexed adults (1-2 weeks old) were weighed and placed in each vial as a group. Before the tests, the insects fasted for 24 hours. Each concentration and control had five replicates. After seven days, four discs and live insects were reweighed. Then the antifeedant parameters were calculated as follows:

•**The Feeding Deterrence Index (FDI) (%)** = [(C - T)/C] *100, where C is the weight of the control diet and T is the weight of the treated diet,

•**Relative growth rate (RGR)=** 100*G/C (G) re-weight gain of insect and (C) weight of food consumed,

•**Relative consumption rate (RCR) =** C/I (C) change in diet weight and (I) starting insect weight, and,

•**The efficiency of conversion of ingested food (ECI) =** 100*G/C (G) re-weight gain of insect and (C) weight of food consumed.

The bio-oil was prepared in dilutions of 500, 1000, 2000, and 3000 mg/g with 5 mL of distilled water. The control was made with only distilled water. Each concentration and control were mixed with 2.5g of wheat. Then the experiment was described above.

Data analysis:

Toxicity data, LC_{50} values, and their upper and lower confidence interval limits, as well as the intercept, were estimated by probit analysis using Ldp line software (Finny, 1971). Significant differences between treatments were determined using non-overlapping, 95% confidence limits (P 0.05). Data from antifeedant bioassays were recorded and expressed as means (standard deviations). The significance of mean differences between treatments and controls was compared statistically using an analysis of variance (ANOVA) at the 0.05% probability level, with individual pairwise comparisons using Tukey's HSD test via Co-Stat software.

RESULTS

Chemical component myrrh, frankincense oil, and bio-oil:

The essential oils of both myrrh and frankincense components were identified by GC–MS. The main constituents of essential oils are listed in Table (1); myrrh oil has 20 components that were considered. The highest component discovered in this oil was Diethyl Phthalate (75.15%). Also, Eugenol (4.88%), Curzerene (4.07). The major constituents in the frankincense oil were alpha-Thujene (30.70%), Octyl acetate (20.44%), Oxirane (O-Cymene) (3.01%), Limonene (2.96%), à-Pinene (2.88%), p-Xylene (2.51%), and Sabinene (2.11%) with a total of 25 compounds constituting 99.58% of EO contents

Table 1. Chemical components of myrrh oil and frankincense essential oils (%)

Components	% Myrrh oil	% Frankincense oil	RT* (min)
alpha-Thujene	-	30.70	4.99
à-Pinene	-	2.88	5.20
p-Xylene	-	2.51	5.71
Sabinene	-	2.11	6.14
-3Carene	-	1.62	6.75
Limonene	-	2.96	7.25
O-Cymene	-	3.01	7.87
Pentylcyclopropane	-	1.26	8.85
Eugenol	4.88	-	10.19
Cyclohexane,1-thenyl-1-methyl-2,4-bis(-methylethnyl)-, [1S-à,2á,4á)]	1.61	-	10.59
Isocaryophyllene	0.59	-	11.09
β-Ionene	1.27	-	12.63
Curzerene	4.07	-	12.77
Octyl acetate	-	20.44	13.18
Estragole	-	1.58	14.96
Diethyl Phthalate	75.51	-	15.73
tau. -Cadinol	1.77	-	15.94
Benzoic acid, 4-(benzoyloxy)-, phenylmethyl ester	2.36	-	18.01
Caryophyllene oxide	1.85	-	29.12
Verticillol	-	1.24	33.12
Oxirane	-	4.73	35.71
cis-Z-à-Bisabolene epoxide	-	9.76	35.77
9-3,3)-dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol	-	8.46	36.04

* RT = retention time (min)

The GC-MS analysis of the *C. sempervirens* bio-oil revealed a total of 40 different components as listed in Table (2), in which the phenolic compounds comprise more than 50% of the components, and the rest are classified as benzene derivatives and organic acids.

Table 2. Chemical composition of the bio-oil from Mediterranean cypress (*Cupressus sempervirens*) trees under investigation.

Compound	% Area	RT* (min)
Acrylic acid	2.92	4.12
6-Oxabicyclo [3.2.1] nonan-7-one,1,3,5-trimethyl-	1.68	4.76
o-Guaiacol	7.23	5.14
m-Cresol	1.37	5.59
2-Cyclopenten-1-one,3-ethyl-2-hydroxy-	1.43	6.16
Creosol	13.36	7.14
1,3-Dioxolane,4-[[(2-methoxy-4-octadecenyl) oxy] methyl]-2,2-imethyl-	2.40	8.06
2,3,4,4-Tetramethyl-1,3-pentanediol	1.37	8.31
4-Isopropyl-5-methyl-4-hexen-1-ol	6.22	8.78
Brenzkatechin	1.23	9.27
Aspidocarpine	1.30	9.76
Formic acid, 2,6-dimethoxyphenyl ester	3.34	10.87
Homocatechol	5.62	11.45
Isoeugenol	7.33	12.39
Methyl vanillyl ether	2.38	12.62
Alpha-cedrenoxid	3.29	13.19
Acetovanillone	5.79	13.90
2-(Trimethylsilyl)benzenethiol	6.20	14.05
Guaiacylacetone	13.79	14.70
Heptanoic acid,4,5-dimethoxy-2-nitrobenzyl ester	2.05	15.53
Benzenepropanol,4-hydroxy-3-methoxy-	2.44	17.20

***** RT = retention time (min)

Toxicity of myrrh oil, frankincense oil, and bio-oil:

The results clearly showed the high toxicity of essential oils, myrrh and Frankincense against *S. oryzae* after 7 days of exposure time (Table 3). Almost the same values of LC_{50} were recorded after seven days of exposure to myrrh oil and Frankincense oil, 728 and 722 mg/kg. bio-oil from *C. sempervirens* Its LC_{50} was less than 300,000 mg/kg after 7 days of exposure time.

Table 3. Toxicity of *S. oryzae* exposed to wheat grains treated with essential oils and bio-oil

Materials	LC50 (mg/kg)	Confidence limits Lower –upper	χ2(df =4)	Slope ± SE
frankincense oil	722	-2698404	2.57	**0.57±0.18**
myrrh oil	728	680- 781	3.41	**5.26±0.66**
bio-oil	3>00,000	-	-	

Combination of the tested oils and bio-oil:

illustrates the mortality percentages of *S. oryzae* adults that were exposed to the individual application of LC_{50} values of EOs and bio-oil at 300,000 mg/kg compared to the mixture of the EOs with bio-oil from *C. sempervirens* after 7 days of exposure time (Figure 1). Bio-oil alone showed very low toxicity against *S. oryzae* even with a high concentration of 300,000 mg/kg as the mortality percentage was recorded to be 14.33%. The same concentration of bio-oil was mixed with LC_{50s} of myrrh and frankincense essential oils. The mixtures showed significantly high efficiency as the mortalities increased to 83.00% and 98.33% with myrrh and frankincense essential oils, respectively. The toxicity of the mixtures of myrrh oil and frankincense oil with bio-oil increased against *S. oryzae* by about 1.91 and 2.27-fold compared to myrrh oil and frankincense oil alone.

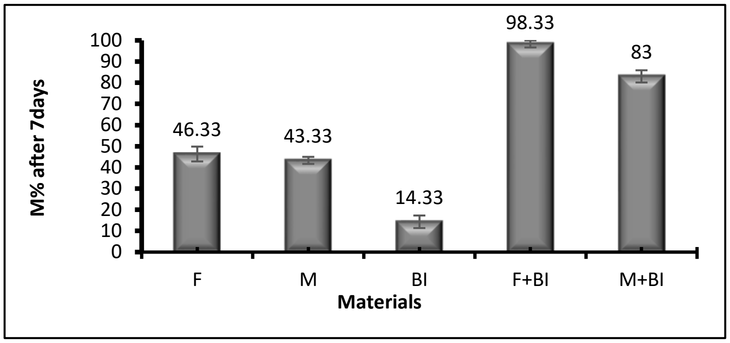

Figure 1. Mortalities percentage of *S. oryzae* adults exposed to LC_{50} myrrh (M) and frankincense (F) essential oils alone and bio-oil (BI) 300,000 mg/kg from *Cupressus sempervirens*, in combination after7days exposure time.

Antifeedant activity:

The antifeedant parameters that were recorded for the tested essential oils showed high nutritional indices and feeding deterrence activity FDI for myrrh oil in all treatments compared to Frankincense oil, and the values of FDI were increased as the concentrations of the oils increased. The FDI values of myrrh oil ranged from 0.00, 16.06%, to 69.76%, which corresponds to doses of 0, 0.01, and 0.125 mg/kg, respectively (df = 6; F = 21.73; p.05; table 3). The results also indicated that the RCR in treated insects decreased significantly (df = 6; F = 10.97; p <0.05) with oil concentration increase, as recorded for the highest concentration to be 0.680 g/g compared with that for the control, 3.14 g/g. RGR values were reduced by 100% and 85.7% in insects fed on treated discs with 0.125 and 0.063 mg/kg of myrrh oil, respectively (table 3). The RGR was significantly reduced (p< .05) at all myrrh oil concentrations and recorded for all treatments to be significantly lower than that recorded for the control diet (df= 6; F= 8.21; p < 0.05). The rate of ECI was reported to be decreased with oil concentrations increasing, and the most reduction was recorded for the highest concentration at 93.13%. There is no significant difference between concentrations of 0.01, 0.016, and 0.032 mg/kg (df = 6; F = 2.81; p <0 .05; Table 4).

The results showed that frankincense oil changed the nutritional indices significantly at all concentrations compared with the control. FDI showed a significant increment in treated adults (df = 6; F = 25.81; p < 0.05). FDI values were increased to 29.18 and 35.89, which corresponded to the concentrations of 0.0125 and 0.063 mg/kg. The RCR fell significantly from 2.72 g to 1.45, 1.62, and 1.67g with 0.00, 0. 125, 0.063, and 0.032mg/kg (df =6; F= 9.76; p < 0.05) and RGR showed a decrease with concentration increase, as recorded for the highest concentration 0.0125mg/g to be 0.1 g (df =6; F= 7.21; p < 0.05) Also, ECI declined to 4.51 % at the highest concentration compared to 12.11% for the control (df = 6; F = 43.84; p < 0.05; table 4).

Table 4. Antifeedant activity of some the essential oils against *Sitophilus oryzae*

Essential oil	**Conc. (mg/g)**	**FDI ±SE**	**Mean nutritional index ±SE**		
			RCR(g)	**RGR(g)**	**ECI (%)**
myrrh	0.0	0.00 (0.00) d	3.14(0.46) a	0.21(0.03) a	**8.01(1.27) a**
	0.005	0.00(01.00) d	3.12(0.17) a	0.14(0.01) ab	**7.41(0.75) a**
	0.01	16.06(4.23) cd	2.07(0.08) ab	0.09(0.02) bc	**5.04(3.44) ab**
	0.016	24.08(4.31) bcd	1.87(0.08) bc	0.07(0.00) bc	**4.09(0.48) ab**
	0.032	24.28(4.78) bc	1.71(0.11) bc	0.06(0.00) bc	**3.90(0.35) ab**
	0.063	45.25(6.34) ab	1.22(0.13) bc	0.03(0.00) c	**3.81(1.23) ab**
	0.125	69.76(10.01) a	0.80(0.22) c	0.00(0.00) c	**0.55(4.32) b**
frankincense	0.0	0.0(0.00) d	2.72(0.41) a	0.32(0.00) a	**12.11(0.94) a**
	0.005	6.20(1.25) cd	2.07(0.08) ab	0.31(0.01) ab	**11.49(0.96) a**
	0.01	11.69(0.45) c	1.67(0.12) b	0.27(0.05) ab	**10.65(0.63) a**
	0.016	16.14(0.95) bc	1.58(0.07) b	0.25(0.03) ab	**8.48(1.15) b**
	0.032	25.00(3.4) ab	1.55(0.3) b	0.20(0.02) abc	**6.41 (0.21) bc**
	0.063	29.18(3.85) a	1.34(0.08) bc	0.18(0.05) bc	**5.67(0.04) c**
	0.125	35.89(4.00) a	0.47(0.063) c	0.10(0.1) c	**4.51(0.31) c**

Values are means ±SE; within each column, means with the same lowercase letter are not significantly different (p > 0.05).

Bio-oil from *C. sempervirens* showed a significantly low antifeedant efficacy compared to both essential oils. In general, the values of RGR, RCR, and ECI significantly decreased, and contrariwise, the FDI increased compared to the control (Table 5). Statistically, the most effective concentration that increased the FDI value was 2000 mg/g, as the FDI index was 78.55 and there was no significant difference between the concentrations of 2000 and 3000 mg/g (df = 6; F = 64.90 $p < 0.05$). Pyroligneous acid caused a reduction in the value of RCR from 3.63g to 0.42 and 0.25g, which corresponds to the concentrations of 0, 2000, and 3000, respectively (df = 6; F = 4.37; $p < 0.05$). No significant difference was recorded in the value of the RCR index between the highest concentrations (2000 and 3000 mg/g). RGR decreased significantly to 0.009 compared to the control, 0.47 (df = 6; F = 58.61; $p < 0.05$). ECI index was reduced to 0.03g compared with 0.13g for the control treatment (df =6; F= 4.52; $p < 0.05$).

Table 5. The antifeedant activity of bio-oil against *S. oryzae*

Conc. (mg/g)	FDI ±SE	Mean nutritional index ±SE		
		RCR(g)	RGR(g)	ECI (%)
0.0	0.00(0.00) d	3.63(0.23) a	0.47 (0.04) a	0.13(0.3)a
500	44.74(7.98) c	1.95(0.62) b	0.13(0.03) b	0.08(0.2) ab
1000	66.16(3.93) b	0.71(0.08) bc	0.04(0.00) bc	0.06(0.2) b
2000	78.55(1.53) ba	0.42(0.03) c	0.02(0.00) bc	0.04(0.00) b
3000	87.94(3.51) a	0.25(0.07) c	0.009(0.00) c	0.03(0.00) b

Values are means ±SE; within each column, means with the same lowercase letter are not significantly different ($p > 0.05$).

DISCUSSION

Essential oil use in insect pest control programs has gained a lot of interest recently because of the disadvantages associated with the improper use of synthetic pesticides. also, the bio-oils produced through the carbonization process of pruning tree residues have both toxic effect and synergistic effects on essential oils (Bruce *et al.,* 2022)

In the present study, the major constituents of myrrh and frankincense oils were analyzed by GC/MS. Myrrh oil has 20 components that were considered. The highest component discovered in this oil was Diethyl Phthalate about 75.51% of the oil contents, which is used in industrial products such as insecticides, repellents, and camphor substitutes (Morteza-Semnani and Saeedi, 2003; Hanuš et al., 2005; Obiezue *et al.,* 2014; Moses *et al*., 2016; Perveen *et al.,* 2018). Also, many researchers found the major component of frankincense oil was alpha-Thujene (Palvela *et al.,* 2021; Kiran *et al.,* 2017). As well, Woolley et al., 2012 reported that a-thujene (7.9%), a-pinene (37.3%), sabinene (4.9%), b-pinene (1.8%), and limonene (14.4%) were present in the chemical composition of the Frankincense oil (Woolley *et al.,* 2012). The present work shows the strong efficacy of the botanical extract (myrrh) which could be used as a grain protection agent. Probit analysis data of bio-oil showed a very low toxic effect. Our results were agreed with many researchers. (Shonouda *et al.,* 2000) revealed the strong efficacy of myrrh extract to control the cotton leafworm. Also, it can be utilized as a biological insecticide on date palm aphids (Al-Keridis, 2022). Some other studies have ascertained the potency of myrrh as an antibacterial, antifungal, antiparasitic, and bio-insecticide natural agent (Allam *et al.,* 2001; Sheir *et al.,* 2001; Perveen *et al*., 2018; Becheker *et al.,* 2022;).The efficiency of myrrh oil may be attributed to its mode of action as Acetyl Choline esterase (AChE) inhibitor as described by (Hussein *et al*., 2019) who found that the botanical extracts resulted from leaves, bark, and resin of C. *myrrha* caused inhibition of AChE activity by 17, 26, and 29% for leaves, bark, and resin respectively.

The chemical composition of frankincense oil studied herein was like previous reports, with some variations due to different growing regions, and genetic and nutritional factors (Mertens *et al.,* 2009). The insecticidal activity reported for Frankincense essential oil in our study towards *S. oryzae* may be related to the abundance of the monoterpene compound, alpha-Thujene. As reported by Jankowska *et al.,* 2017 that monoterpenes have neurotoxic effects on insects that may result from their Conjugation with different target sites such as AChE, Octopamine receptors, and GABA receptors (Jankowska *et al.,* 2017). Aliphatic ester octyl acetate is the second major component of frankincense oil which was reported by Zangerl et al 2012 to have insecticidal activity against Webworms (Zangerl *et al.,* 2012). Similar supportive studies have explored the insecticidal efficacy of frankincense oil against *Culex quinquefasciatus*, *M. domestica*, *S. littoralis* the pulse beetles *C. chinensis* and *C. maculatus, Aedes aegypti, A. stephensi* (Kiran *et al.*, 2017; Amer *et al.,* 2006; Pavela *et al*., 2021). other than that, bio-oil from *C. sempervirens* showed in general weak activity.

The GC-MS analysis of the *C. sempervirens* bio-oil revealed a total of 40 different components in which the phenolic compounds comprise more than 50% of the components, and the rest are classified as benzene derivatives and organic acids, the chemical profile of *C. sempervirens* bio-oil is similar to that prepared from *Toona Sinensis* wood which contains mainly carboxylic acids, phenolic compounds and other benzene derivatives (Adfa *et al*., 2017)

Another study of the chemical composition of bio-oil derived from *Cinnamomum parthenoxylon* revealed that organic acids and phenols were the main components (Adfa *et al.* 2020). When applied alone, the toxicity data revealed that bio-oil had a low efficiency.

The toxicity of the mixtures of myrrh oil and frankincense oil with bio-oil increased against *S. oryzae* by about 1.91 and 2.27-fold compared to myrrh oil and frankincense oil alone. The synergistic effect of bio-oil may be due to its chemical components, of which phenolic compounds comprise more than 50% and the rest are classified as benzene derivatives and organic acids. The toxicity results showed the low activity of bio-oil when applied alone, which agrees with the results of the study conducted by Kim et al 2008, who reported that the insecticidal activity of bio-oil was low against two species of planthoppers, but significantly increased when mixed with carbosulfan, (Kim., *et al* 2008) Similar to the work of Hashemi et al 2014 who found that the individual application of bio-oil on cigarette beetle *Lasioderma serricorne* didn't exhibit insecticidal activity while the mortality greatly increased when mixed with the methanol extract of *Salvia leriifolia* (Hashemi *et al.,* 2014). According to Pangnakorn and Kanlaya 2014, the mixture of bio-oil and citronella extract gave the highest mortality against the housefly (Pangnakorn and Kanlaya, 2014). The mixture of bio-oil with essential oils could be recommended as formulations of potential plant-based insecticides, which are effective even at low dosages of essential oils and is a viable alternative resource to synthetic insecticides. However, further studies are required to develop advanced techniques to improve the efficacy, and stability of these mixtures.

myrrh oil showed high nutritional indices and FDI compared to Frankincense oil. The higher the FDI value, the stronger the antifeedant activity and the lower the adult food consumption. Bio-oil from *C. sempervirens* showed a significantly low antifeedant efficacy compared to both essential oils. These results are similar to those of Shonouda *et al*., 2000, who reported several biological disturbances in the development of *S. littoralis* larvae that were treated with myrrh extract. (Shonouda *et al*., 2000). After being treated with myrrh extract, the larvae of *Lucilia sericata* developed various malformations, including small-sized, contractile, and damaged larvae; the small size of pupa; incomplete emergency of the small-sized, malformed adult; and poorly developed (Hoda *et al*., 2016). Many researchers estimated the antifeedant activity of frankincense oil against insects. For example, the %FDI of frankincense oil against *C. maculatus* and *C. chinensis* was 82 and 74.31% at 0.066 µl/ml (Kiran *et al.*, 2017). The most abundant ester that is found in frankincense oil and the reproductive structures of plants is Octyl acetate, which plays a very important role as a feeding deterrent to webworm larvae and acts as a larval attractant (Zangerl *et al.,* 2012).

Bio-oil inhibited subterranean termite feeding significantly, according to Kadir *et al.,* 2022 (Kadir *et al.,* 2022). Isoeugenol, according to (Huang *et al.,* 2002), reduced RGR and RCR in adults of *Sitophilus zeamais* and *Tribolium castaneum*, as well as ECI in *T. castaneum*. Another study conducted by Leonor et al 2018 to evaluate the larvicidal and antifeedant activity of 15 semisynthetic eugenol derivatives against *Spodoptera frugiperda*, found that isoeugenol showed good antifeedant activity with a high antifeedant index (70–78%) (Vargas-Méndez *et al.,* 2019). Essential oils and bio-oil were proven to exhibit not only their insecticidal activity but also their roles in reducing the feeding deterrent and development of many stored product insects.

CONCLUSION

Based on our results, we can conclude that wood vinegar prepared from *C. sempervirens* can be used effectively to synergize other active compounds such as essential oils consequently, the used concentrations of EOs will be decreased. Also, the results show the potential antifeedant activity of bio-oil and the high antifeedant activity of both EOs, myrrh and frankincense oils against *S. oryzae.*

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